AD_____

Award Number: W81XWH-06-1-0108

TITLE: Stromal Mesenchyme Cell Genes in Prostate Cancer Development: Epigenetic Markers for Cancer and Potential Targets for Therapy

PRINCIPAL INVESTIGATOR: Young Ah Goo, Ph.D.

CONTRACTING ORGANIZATION: University of Washington
Seattle WA 98105

REPORT DATE: December 2007

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-12-2007	Final	12 Nov 05 – 14 Nov 07

4. TITLE AND SUBTITLE
Stromal Mesenchyme Cell Genes in Prostate Cancer Development: Epigenetic Markers for Cancer and Potential Targets for Therapy

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-06-1-0108

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Young Ah Goo, Ph.D.

E-Mail: youngah@u.washington.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Washington
Seattle WA 98105

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT Functional development of the prostate is governed by stromal induction and epithelial response. Given the importance of this communication, it is possible that cancer could arise from a loss of this communication. We carried out comparative proteomic and transcriptome analyses to identify organ-specific stromal signaling factors from prostate and bladder stromal cells. Secreted proteins were identified by glycopeptide-capture followed by mass spectrometry. Protein quantification was done by PICA (Peptide Ion Current Area) method. A list of 116 prostate and 84 bladder glycoproteins was identified. The prostate proteins include cathepsin L, neuroendocrine convertase, and tumor necrosis factor receptor involved in signal transduction, extracellular matrix interaction, and differentiation. For the transcriptome analysis, cells were isolated by CD49a for prostate and CD13 for bladder. In a comparative analysis, 91 bladder and 288 prostate genes were identified. Further subtractive analysis resulted in a list of 50 prostate stromal cell-specific genes. Interesting genes were CNTN1, SPOCK3, and MAOB, which are known to be involved in cell interactions and organogenesis. Expression analysis showed that these genes are down-regulated in cancer, suggesting their function is required in normal development.

15. SUBJECT TERMS
stromal signaling molecules, prostate and bladder differentiation, microarray gene expression analysis, glycopeptide-capture method, mass spectrometry

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	64	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

INTRODUCTION

The functional development of the prostate is governed by stromal mesenchyme induction and epithelial response. This stromal/epithelial interaction was demonstrated by heterotypic tissue recombinants engrafted in animal hosts in which the stromal element dictated the organogenesis of the implanted epithelial component. For example, adult human bladder epithelial cells can be transdifferentiated into prostatic structures by prostate inductor. Besides epithelial/stromal interaction, morphogenesis and functional cytodifferentiation are dependent on interactions between epithelium and basement membrane and the extracellular matrix. Prostate development is also under hormonal control and the influence of androgen is primarily mediated by the stromal cells. We postulate that organ-specific stromal cell genes are important factors in organ development. One approach to gain a molecular understanding of epithelial/stromal interaction is to identify, as a first step, the organ-specific stromal signaling factors. We have identified candidate genes responsible for the organ-specific induction of epithelial differentiation through a comparative transcriptome analysis between sorted prostate stromal and bladder stromal cells. Differentially expressed genes by the two stromal cell types were verified with quantitative RT-PCR analysis. Given the importance of stromal cells in epithelial differentiation, it is possible that diseases such as cancer of the epithelial cells could arise from defects in or a loss of stromal influence. We, therefore, examined the expression of prostate stromal cell genes in cancer. Furthermore, stromal signaling factors are made when the stromal cells are grown in culture, since epithelial induction can be achieved with stromal cell conditioned media. As such, quantitative proteomic analysis was carried out to identify differentially expressed secreted proteins found in the culture media of prostate and bladder stromal cells.

BODY

A. Identification of differentially expressed stromal mesenchyme cell genes between the prostate and bladder using isolated cell populations

A.1. Isolation of single cell populations by their specific CD markers using a bulk isolation method with antibody-coated magnetic beads (MACS) and by laser-capture microdissection (LCM)

Our CD phenotyping result showed a layer of stromal cells beneath the bladder urothelium that was positive for CD13 whereas the stromal cells next to the prostatic epithelium were not (Fig. 1). This comparative analysis was therefore expected to reveal differences in gene expression between the two stromal cell types, and most, if not all, of the differentially expressed genes that might play a role in the prostate or bladder differentiation program. For comparative analysis, we isolated single cell populations by the use of CD49a for prostate and CD13 for bladder stromal cells. Prostate and bladder tissue specimens were obtained from Department of Urology tissue acquisition program. For sorting, tissue samples weighing between 1-10 g are minced and digested by collagenase in RPMI1640 supplemented with 5% FBS and 10^{-8} M DHT.[1] The resultant single cells are partitioned on discontinuous Percoll density gradients.

Cells banding at the stromal cell density (□=1.035) are collected as STROM, and cells banding at the epithelial cell density (□=1.07) are collected as EPI. $CD49a^+$ cells are sorted by MACS from STROM. $CD13^+$ cells are sorted from bladder STROM. In MACS, cells are resuspended in 0.1% BSA-HBSS and labeled with the appropriate phycoerythrin (PE)-conjugated antibodies. After the primary antibody, the cells are incubated with anti-PE microbeads. The cell suspension are filtered and sorted by AutoMACS using the POSITIVE SELECTION DOUBLE SORT program (Miltenyi Biotec). Both the positive and negative fractions are analyzed by flow cytometry to assess purity. We use MACS because of the shorter sort time (~30 min) compared to FACS (Fluorescence Activated Cell Sorting) in order not to compromise cell viability. The sorted cells are lysed for RNA preparation and the RNA quality is checked by BioAnalyzer (Aglient technologies).

For LCM, eight □m thick sections of frozen tissue blocks were prepared, immediately fixed in cold 95% ethanol, briefly stained with hematoxylin using Arcturus HistoGene Staining Solution and dehydrated in 100% ethanol followed by xylene, as described in the Arcturus HistoGene LCM Frozen Section Staining Kit protocol. Around 5,000 stromal cells were captured using Arcturus PixCell II. Following microdissection, captured cells were lysed in Arcturus RNA Extraction Buffer. RNA was isolated using Arcturus PicoPure RNA Isolation and treated with RNase-Free DNase. The RNA was amplified by two rounds using Ambion MessageAmp aRNA. RNA sample quality and quantification were assessed by BioAnalyzer then used for array analysis.

This step has accomplished Statement of Work: Task 1.a. and 1.b.

A.2. Array analysis of the sorted cells by Affymetrix oligonucleotide microarrays and validation of differential expression by qRT-PCR

Gene expression was profiled by the Affymetrix human gene-chip arrays. The UG-133 Plus 2.0 gene chip contains probe sets representing 54,675 genes, splice variants, and ESTs. Analyzed were: $CD49a^+$ prostate stromal cells, *vs*. $CD13^+$ bladder stromal cells; prostate LCM stromal cells, *vs*. bladder LCM stromal cells. In using the arrays, total RNA was reverse transcribed with a oligo(T) primer/T7 promoter to produce cDNA. Second-strand cDNA was then synthesized. An in vitro transcription was performed in the presence of biotinylated ribonucleotides. The labeled cRNA was hybridized to the arrays, washed and stained with streptavidin-PE using Affymetrix FS-450 fluidics station, and data was collected with GeneChip Scanner 3000. The Affymetrix data was analyzed with GeneChip Operating Software (GCOS). Scanned images of the arrays were converted to numerical data by GCOS and outputted to tab delimited text files containing Affymetrix probeset ID, signal, present or absent detection call, and detection P-value fields for each probe set. The raw Affymetrix data was filtered to mask genes with signal intensities greater the background threshold. Four or more biological replicates were used for array analysis for bladder and prostate respectively. For differential gene expression detection, the dataset was analyzed by HTself, a self-self based statistical method for microarrays.[2] To apply this method, all possible combination of pair-wise comparisons among experiments were taken to create sets of ratios, similarly as in Okamoto et al.[3] The statistical method used virtual self-self experiments created from actual replicates to derive intensity-dependent cutoffs. Accordingly, a probeset was considered significantly

differentially expressed if all its log-ratio combinations were outside the 99.5% credibility intensity-dependent cutoff.

In the comparative analysis, 91 bladder and 288 prostate differentially expressed genes were identified (Fig. 2). Expression of 4 most differentially expressed genes from the bladder and prostate was validated by qRT-PCR (Fig. 3). For the prostate stromal cells, genes found in the other cell type-specific datasets (CD104 basal, CD26 luminal, and CD31 endothelial cells) were subtracted. Five replicates from each non-CD49a cell types were analyzed and if a gene is present in 3 and more replicates it was considered as "present" and was subtracted from the CD49a sorted cell genes. This resulted in a list of 50 stromal cell-specific genes with 39 known functions. Expression of these 39 genes across the sorted cells is shown in figure 4. The grayscale indicates normalized Affymetrix signal intensity. Signals less than 10 are represented in white and signals greater than 10,000 in black. Higher Affymetrix signal (more black) indicates greater levels of gene expression. Overall, their expression was shown to be enriched in sorted and LCM stromal cells. Some of interesting findings were CNTN1 (contactin 1), which is known to mediate cell surface interactions during neural system development by signaling between axons and myelinating glial cells via its association with CNTNAP1. It function as a GPI-anchored molecule on the cell memebane.[4] SPOCK3 (sparc/osteonectin, testican 3) encodes a secreted protein that participates in diverse steps of neurogenesis and inhibits the processing of pro-matrix metalloproteinase 2 (MMP-2). It may interfere with tumor invasion.[5] MAOB (monoamine oxidase B) catalyzes the oxidative deamination of biogenic and xenobiotic amines and has important functions in the metabolism of neuroactive and vasoactive amines in the central nervous system and peripheral tissues.[6] MAOB expression has been linked to early onset of Parkinson's disease.

Since both cell sorting and LCM methods were used for this project we present our data regarding the efficiency of each method in generating array results. Either method has its own drawback. Unlike cell sorting, the number of cells collected by LCM is often limited so that RNA amplification is necessary to yield sufficient probe concentration for arrays. LCM may also be prone to contamination by cell types not apparent to the LCM operator, for example, infiltrating lymphoid cells in the prostatic epithelium. In flow sorting, there is always the possibility of contamination of non-targeted cell types having the CD molecules used. However, in our hands, the same amount of RNA from laser-captured cells detected only half the number of genes as that by sorted cells. For example, 5,000 prostate stromal cells picked by LCM and 5,000 sorted $CD49a^+$ stromal cells were analyzed. The comparative data obtained is presented by the Venn diagram in figure 5. Using the threshold of signal level of 50, 54% (12272/22743) of the probesets were not detected by the LCM cells compared to 0.9% (210/22743) not detected by sorted cells. Despite this unfavorable comparison LCM may still be the only viable method to obtain certain tumor cell populations for analysis. For many tumor specimens, their size is too small for collagenase digestion to yield enough cells for sorting.

This step has accomplished experimental parts of the Statement of Work: Task 1.c. and 1.d.

Task 1 has been finished.

B. Identification of differentially expressed secreted proteins of stromal cells by quantitative proteomic analysis

B.1. Prostate and bladder stromal cell culture and cell-free conditioned media preparation

Cultures were started by plating single cells prepared by tissue digestion with collagenase as described previously.[7] Briefly, prostate or bladder stromal cells from Percoll gradients were cultured in RPMI-1640 supplemented with 10% FBS and 10^{-8} M DHT. Cells were serially passaged. Light microscopy was used to check cell morphology. Cells were monitored for expression of smooth muscle actin (□SMA) and non-expression of epithelial cell-specific glycoprotein (EGP) by RT-PCR. Percoll gradients were used additionally to remove any contaminant epithelial cell types at serial passages. Prostate and bladder stromal cells (10^7 cells) at the third or fourth passage were placed in RPMI-1640 without FBS and cultured for 24 h. This duration was based on our previous experiments that showed no induction of serum deficiency shock proteins within this time period (unpublished data). The serum-free media treatment was called for because the FBS supplement would overwhelm proteomic analysis with bovine albumin and other abundant serum proteins. The media was made cell-free by centrifugation and filtration. Proteins in the media were concentrated using Centricon Plus-20 (NMWL: 5000, Millipore, Billerica, MA). The tissue digestion media was also analyzed.

This step has accomplished Statement of Work: Task 2.a.

B.2. Identification of differentially expressed secreted glycoproteins

Secreted proteins were identified using the *N*-linked glycoprotein-capture method.[8] Briefly, 1 mg protein from each sample was exchanged into coupling buffer (100 mM NaAc and 150 mM NaCl, pH 5.5) using a desalting column (Bio-Rad, Hercules, CA), and oxidized by adding 15 mM sodium periodate at room temperature for 1 h. After removal of the oxidant with a desalting column, the sample was conjugated to hydrazide resin (Bio-Rad) at room temperature for 10–24 h. Non-glycosylated proteins were removed by washing the resin three times with 1 ml of 8 M urea, 0.4 M NH_4HCO_3, pH 8.3. After the last wash, proteins were reduced in 8 mM Tris-(2-carboxyethyl) phosphine at room temperature for 30 min and alkylated in 10 mM iodoacetamide for 30 min. The resin was washed twice with 1 ml 0.1 M NH_4HCO_3, trypsin was added at 1 mg/200 mg protein for digestion at 37°C overnight. The trypsin-cleaved peptides were removed, and the resin was washed three times successively each in the following solutions: 1.5 M NaCl, 80% acetonitrile (ACN), 100% methanol, water, and 0.1 M NH_4HCO_3. *N*-Linked glycopeptides were released from the resin by 0.3 µl peptide-N-glycosidase F (PNGase F, 500 units/µl, New England Biolabs, Beverly, MA) at 37°C overnight. Released peptides were dried and resuspended in 50% ACN, 0.1 % formic acid for MS analysis. Four experimental replicates each for prostate and bladder were analyzed by LCMS using a Michrom Paradigm MS4B HPLC system (Michrome Bioresources, CA) that was coupled via electrospray ionization (ESI) on-line to a linear ion trap (LTQ) Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (LTQ-FT, ThermoFinnigan, CA).

Glycopeptides were fractionated by online C18 reverse phase cation-exchange chromatography into four fractions, and each fraction was analyzed. Acquired MS/MS spectra were searched for sequence matches against the International Protein Index (IPI) human protein database (version 3.01) using SEQUEST. PeptideProphet and ProteinProphet, which compute a probability likelihood of each identification being correct, were used for statistical analysis of search results.[9] A PeptideProphet probability score of ≥ 0.5 was chosen as a filter which corresponds to an error rate of ≤ 3%. For proteins, a ProteinProphet probability score of ≥ 0.9 was applied which corresponds to less than a 0.02% error rate. To reduce the false positive rate further, peptides were filtered for the N-linked glycosylation motif (N-X-S/T where X is any amino acid except proline). The study showed identification of a smaller number of proteins (presumably due to more complex sample dynamic range) and increased detection of intracellular proteins from the tissue digestion media than the cell-free media of cultured cells. Since the purpose of the study is to identify secreted proteins, future investigation was focused on using cell-free conditioned media.

Protein expression was estimated by a novel isoptope-free quantification software, PICA (peptide ion current area, Ryu, S., *et al*., in press). Briefly, the expression levels of protein were estimated by using peak intensity area.[10,11] After background noise subtraction, the peak intensities were normalized using total ion intensity. All peptides identified for a given protein were collected, and the theoretical *m*/*z* of first three isotope peaks of a peptide was calculated. Peaks with 3 or more scans to reconstruct an ion chromatogram were extracted in the study. The protein expression was estimated by summing the peak areas of its corresponding peptides. The protein ratio between bladder and prostate was derived by dividing an average protein expression value of bladder by the average protein expression value of prostate. Differentially expressed proteins were identified by two-sample *t*-test (with unequal variance), and a false discovery rate (FDR) was measured to correct for multiple testing variations.

A total of 116 glycoproteins were identified in the media of prostate stromal cell cultures and, of these, 34 were identified in the prostate but not the bladder. A total of 84 glycoproteins were identified in the media of bladder stromal cell culture and 2 of these were identified in the bladder but not the prostate. The two secreted proteomes thus shared expression of 82 proteins (Fig. 6). Caution is needed in these types of comparisons because proteins identified in one sample only may be due to under-sampling by data-dependent ion selection,[12] data filtering, or peptides that failed to generate the necessary 3 isotopic peaks for inclusion for quantification.

Identified glycoproteins were annotated with Gene Ontology (GO)[13], which assigns putative cellular compartmentalization and molecular functions. Not surprisingly, >75% of the proteins identified by the glycopeptide-capture method were annotated as secreted or membrane-associated molecules (Fig. 7). Protein- and ion-binding functions, hydrolase activity (catalytic hydrolysis of C-O, C-N, C-C, phosphoric anhydride bonds, etc.) were the most common ascribed to these secreted proteins (Fig. 8). Both bladder and prostate proteins exhibited a similar categorization in their GO functions. The binding, signal transduction, and transport functions could all be characteristic for those involved in intercellular communication.

A total of 29 proteins with FDR ≤ 0.05 were considered to be statistically significant for differential expression between prostate and bladder (Table 1). Only three of these 29 proteins were annotated in GO as either intracellular or unknown, all others were as secreted or membrane-associated cell surface proteins. When queried for GO biological processes, these proteins were found to be involved predominantly in physiology, cell adhesion, communication, and development (Fig. 9). Of these differentially expressed proteins, quantitative proteomic

analysis found that 24 were over-expressed in the prostate and 5 in the bladder. Of these, CTSL was the most differentially expressed protein in prostate stromal cells. In the literature, this lysosomal cysteine protease is reported to be synthesized in large amounts and secreted by tumor cells in culture. Tumor CTSL activity was proposed to facilitate invasion and metastasis by its degradation of ECM (Extra Cellular Matrix) components.[14-16] An elevated CTSL level has been observed in primary cell cultures from tumors.[17] Prostate stromal CTSL may well be one of the factors in tumor cell promotion. For the bladder proteins, the most differntially expressed APOH (β-2-glycoprotein I, apolipoprotein H) appears to prevent activation of the intrinsic blood coagulation cascade by binding to phospholipids on the surface of damaged cells.

The finding that the majority of the differentially expressed secreted proteins were proteases, kinases, and growth factors involved in protein binding, transport and/or signal transductions suggests their possible involvement in stromal and epithelial communication, development, and cell differentiation. Stromal secretion of cytokines, growth factors, and proteases is essential for epithelial differentiation and morphogenesis. Some of these factors are also involved in reciprocal stromal/epithelial interaction in prostate cancer.[18] When the secreted proteins were analyzed for their pathway involvement, several proteins in ECM-receptor interaction pathways were upregulated in prostate stromal cells relative to bladder stromal cells. This suggests stromal/epithelial signaling may be accomplished through transmission via activation of the ECM-receptor pathway in prostate. With the identification of secreted stromal proteins, one can begin to study their biological function and we are developing an in vitro assay system to test the functional property of these identified proteins.

This step has accomplished Statement of Work: Task 2.b. and 2.c.

B.3. Validation of differential expression by Western blot

Of the 29 differentially expressed proteins, quantitative proteomic analysis found that 24 were over-expressed in the prostate and 5 in the bladder. Their differential expression was validated by Western blot analysis (Fig. 10). The limitation was that for some proteins, either no antibody was available or both antibodies failed to detect the targets. Detection failure could be due to many reasons, among which could be antibody quality, protein structure, amount of accessible antigen, etc. Nevertheless, for those that were detected, their MS derived differential expression between prostate and bladder was verified. The CD90 antibody was used to control for equal gel loading of protein. CD90 is a GPI-anchored protein found released into the culture media by stromal cells, and the expression of CD90 in cultured bladder and prostate stromal cells has been previously reported.[7]

This step has accomplished Statement of Work: Task 2.d.

Task 2 has been finished. A manuscript is currently being reviewed by a journal.

C. Stromal genes expression analysis in cancer

We hypothesized that cancer could be due to defects in stromal/epithelial interaction, and expression of stromal genes like CNTN1, SPOCK3, and MABO could be altered in cancer. Gene expression analysis of CNTN1, SPOCK3, and MABO in patient-matched normal/non-cancer and cancer was analyzed by RT-PCR. Tumor specimens were excised from cancer foci in surgically resected glands, and non-cancer specimens were taken from cancer-free areas. The small pieces of tissue (~ 1 mm^3) were homogenized, and RNA was precipitated by isopropanol for CP (prostate cancer or enriched for cancer) and NP (normal prostate) cDNA synthesis as described in a previous report.[19] The small size of the tissue was to ensure a purer sample of cancer without non-cancer tissue. Primers for smooth muscle actin (αSMA) were used to monitor the suitability of the cDNA for analysis. In addition to primary cancer, bone, liver, and lymph node metastasis specimens were obtained from autopsies of donor patients. For this experiments, processed cDNA from these specimens were obtained through the Department of Urology's tumor acquisition program. In fig. 11, CNTN1 was detected in all four NP samples, but its level was either decreased or undetectable in the CP and metastasis samples (expression of αSMA was used as a control for the representation of stromal cells in NP and CP). MAOB and SPOCK3 were other genes of interests identified through array analysis. They also showed similar expression patterns in the same samples even though differential expression between NP and CP was not as striking as that of CNTN1.

Our hypothesis that cancer or disease could arise from defects in stromal/epithelial communication and expression of key genes could be altered in cancer was shown to be the case for CNTN1. We are currently developing an in vitro system to test the functional property of these identified stromal genes.

This step has accomplished Statement of Work: Task 3.a. and 3.b.

Task 3 has been finished. A manuscript summarizing studies conducted in Task1 and Task3 is currently in preparation.

KEY RESEARCH ACCOMPLISHMENTS

A. Isolated prostate stromal cells by $CD49a^+$ cell sorting and LCM, and created prostate stromal cell-specific transcriptomes

B. Isolated bladder stromal cells by $CD13^+$ cell sorting and LCM, and created bladder stromal cell-specific transcriptomes

C. Identified differentially expressed stromal genes between bladder and prostate and validated differential expression by qRT-PCR

D. Identified secreted proteins of prostate and bladder stromal cells from the conditioned media by glycopeptide-capture proteomics

E. Identified differentially expressed secreted proteins between prostate and bladder stromal cells and validated differential expression by Western blot analysis

F. Examined the expression of prostate stromal cell genes in cancer.

REPORTABLE OUTCOMES

Goo, YA., Ryu, S., Walashek, L., Shaffer, S., Liu, AY., Goodlett, DR. Comparative analysis of secreted proteins of human prostate and bladder stromal mesenchyme cells. 7th Siena Meeting: From Genome to Proteome. Siena, Italy Oct 2006, Poster presentation

Goo, YA., Ryu, S., Shaffer, S., Walashek, L., Liu, AY., Goodlett, DR. Secreted glycoproteins of human prostate and bladder stromal mesenchyme cells. USHUPO 3rd Annual Conference. Seattle, USA Mar 2007, Poster presentation

Goo, YA., Liu, AY., Goodlett, DR. Identification of human prostate and bladder stromal factors by quantitative transcriptome and proteomics analysis. CDMRP PCRP 2007 IMPaCT Meeting. Atlanta, USA Sep 2007, Poster presentation

Goo, YA., Liu, AY., Goodlett, DR. Identification of human prostate and bladder stromal cell signaling factors by quantitative proteomic and transcriptome analysis. HUPO 6th Annul World Congress. Seoul, Korea Oct 2007, Poster presentation

CONCLUSION

We have identified a number of candidate prostate-specific stromal genes that might be involved in stromal/epithelial signaling through a comparative transcriptome analysis between sorted or LCM prostate and bladder stromal cells. Expression of the stromal genes was altered in cancer suggesting their possible role in cancer development. Furthermore, most of these products are thought to be made when stromal cells are in culture because epithelial induction can be achieved with stromal cell conditioned media. Quantitative proteomic analysis using the glycopeptide-capture method was carried out to identify secreted proteins. The label-free proteomic analysis identified a number of secreted proteins which may be involved in stromal/epithelial signaling and organ-specific differentiation.

REFERENCES

1. Liu, A. Y., True, L. D., LaTray, L., Nelson, P. S., *et al.*, Cell-cell interaction in prostate gene regulation and cytodifferentiation. *Proc Natl Acad Sci U S A* 1997, 94, 10705-10710.
2. Vencio, R. Z. and Koide, T., HTself: self-self based statistical test for low replication microarray studies. *DNA Res* 2005, 12, 211-214.
3. Okamoto, O. K., Carvalho, A. C., Marti, L. C., Vencio, R. Z. and Moreira-Filho, C. A., Common molecular pathways involved in human CD133+/CD34+ progenitor cell expansion and cancer. *Cancer Cell Int* 2007, 7, 11.
4. Reid, R. A., Bronson, D. D., Young, K. M. and Hemperly, J. J., Identification and characterization of the human cell adhesion molecule contactin. *Brain Res Mol Brain Res* 1994, 21, 1-8.
5. Nakada, M., Yamada, A., Takino, T., Miyamori, H., *et al.*, Suppression of membrane-type 1 matrix metalloproteinase (MMP)-mediated MMP-2 activation and tumor invasion by testican 3 and its splicing variant gene product, N-Tes. *Cancer Res* 2001, 61, 8896-8902.
6. Binda, C., Newton-Vinson, P., Hubalek, F., Edmondson, D. E. and Mattevi, A., Structure of human monoamine oxidase B, a drug target for the treatment of neurological disorders. *Nat Struct Biol* 2002, 9, 22-26.
7. Liu, A. Y., LaTray, L., van den Engh, G., Changes in cell surface molecules associated with in vitro culture of prostatic stromal cells. *Prostate* 2000, 45, 303-312.
8. Zhang, H., Li, X. J., Martin, D. B. and Aebersold, R., Identification and quantification of N-linked glycoproteins using hydrazide chemistry, stable isotope labeling and mass spectrometry. *Nat Biotechnol* 2003, 21, 660-666.
9. Goo, Y. A., Yi, E. C., Baliga, N. S., Tao, W. A., *et al.*, Proteomic analysis of an extreme halophilic archaeon, Halobacterium sp. NRC-1. *Mol Cell Proteomics* 2003, 2, 506-524.
10. Radulovic, D., Jelveh, S., Ryu, S., Hamilton, T. G., Foss, E., Mao, Y., Emili, A., Informatics platform for global proteomic profiling and biomarker discovery using liquid chromatography-tandem mass spectrometry. *Mol Cell Proteomics* 2004, 3, 984-97.
11. Ryu, S., Radulovic, D., Gallis, B., Goo, Y. A., Shaffer, S. A., Goodlett DR: Comparison of Isotope Coded Affinity Tag (ICAT) to Regular Old Ordinary Mass Spectrometry (ROOMS) for Quantitative Proteomics. *2005 ASMS POSTER* ThP32-521.
12. Yi, E. C., Marelli, M., Lee, H., Purvine, S. O., *et al.*, Approaching complete peroxisome characterization by gas-phase fractionation. *Electrophoresis* 2002, 23, 3205-3216.
13. Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., *et al.*, Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet* 2000, 25, 25-29.
14. Chauhan, S. S., Goldstein, L. J. and Gottesman, M. M., Expression of cathepsin L in human tumors. *Cancer Res* 1991, 51, 1478-1481.
15. Staack, A., Tolic, D., Kristiansen, G., Schnorr, D., *et al.*, Expression of cathepsins B, H, and L and their inhibitors as markers of transitional cell carcinoma of the bladder. *Urology* 2004, 63, 1089-1094.
16. Shuja, S., Sheahan, K. and Murnane, M. J., Cysteine endopeptidase activity levels in normal human tissues, colorectal adenomas and carcinomas. *Int J Cancer* 1991, 49, 341-346.
17. Friedrich, B., Jung, K., Lein, M., Turk, I., *et al.*, Cathepsins B, H, L and cysteine protease inhibitors in malignant prostate cell lines, primary cultured prostatic cells and prostatic tissue. *Eur J Cancer* 1999, 35, 138-144.

18. Chung, L. W., Huang, W. C., Sung, S. Y., Wu, D., *et al.*, Stromal-epithelial interaction in prostate cancer progression. *Clin Genitourin Cancer* 2006, 5, 162-170.

19. Liu, A. Y., Corey, E., Vessella, R. L., Lange, P. H., True, L. D., Huang, G. M., Nelson, P. S., Hood, L., Identification of differentially expressed prostate genes: increased expression of transcription factor ETS-2 in prostate cancer. *Prostate* 1997, 30,145-153.

SUPPORTING DATA

Figure 1. CD13 immunohistochemistry of the prostate and bladder. CD13 stains luminal epithelial cells (black arrow) of prostatic glands as shown on the left. In the bladder, CD13 stains a subpopulation of stromal cells (black arrow) in the lamina propria as shown on the right. The partially denuded urothelium is indicated by the red arrow. Magnification is 40x.

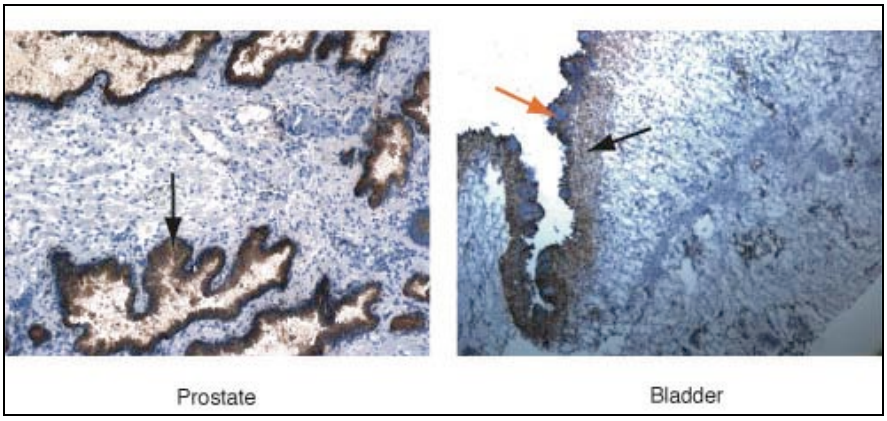


Figure 2. Distribution of differentially expressed stromal genes in bladder/prostate. Up-regulated genes in the bladder (A) and the prostate (B) are shown in red.

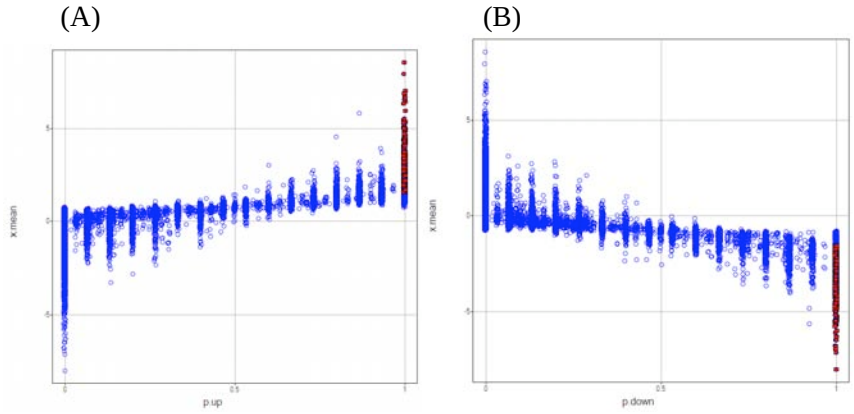


Figure 3. Quantitative real-time RT-PCR expression analysis. SPOCK3: (sparc/osteonectin proteoglycan, testican 3), MSMB (β microseminoprotein), CXCL13 (chemokine ligand 13), PAGE4 (P antigen family member 4) are upregulated in prostate stromal cells while TRPA1 (transient receptor potential cation channel), HSD17B2 (hydroxysteroid 17-β dehydrogenase 2), IL24 (interleukin 24), SALL1 (sal-like 1) are up regulated in bladder stromal cells.

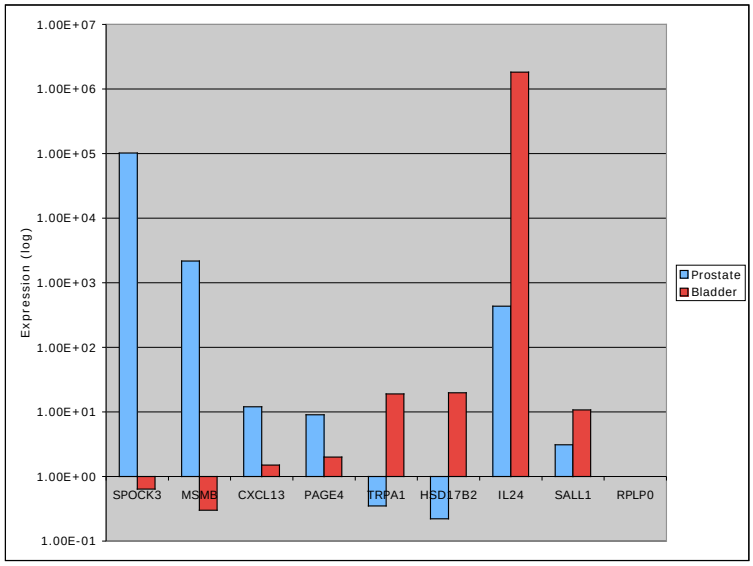
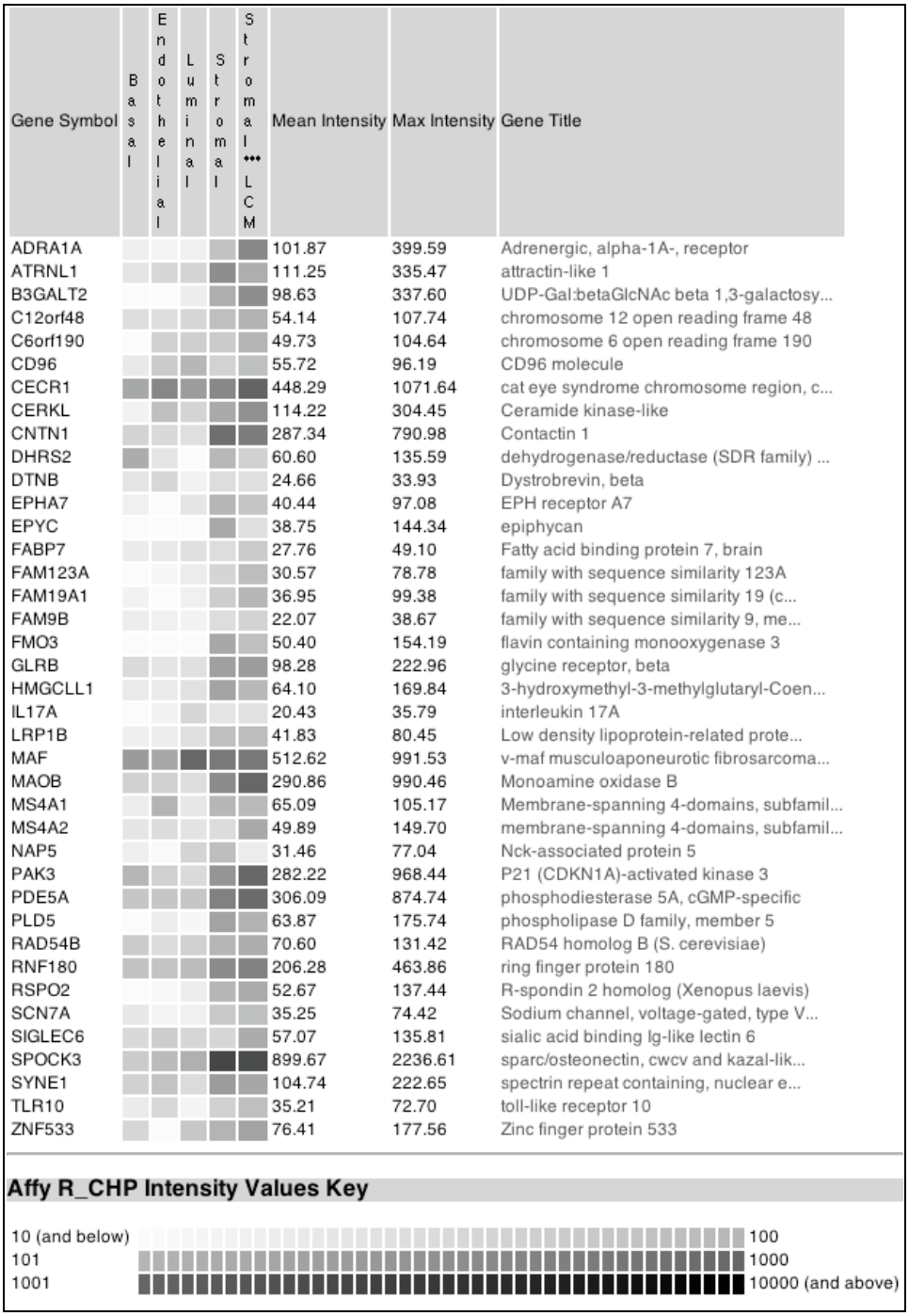


Figure 4. Expression profile of prostate stromal cell-specific genes. "Basal" is CD104-sorted cells, "Endothelial" is CD31-sorted cells, "Luminal" is CD26-sorted cells, "Stromal" is CD49a-sorted cells, and "stromal LCM" is LCM cells. "Mean Intensity" is the average intensity of the gene searched across the four cell types. "Max Intensity" is the highest signal for the gene searched in the 4 cell types.



Gene Symbol	Basal	Endothelial	Luminal	Stromal	Stromal *** LCM	Mean Intensity	Max Intensity	Gene Title
ADRA1A						101.87	399.59	Adrenergic, alpha-1A-, receptor
ATRNL1						111.25	335.47	attractin-like 1
B3GALT2						98.63	337.60	UDP-Gal:betaGlcNAc beta 1,3-galactosy...
C12orf48						54.14	107.74	chromosome 12 open reading frame 48
C6orf190						49.73	104.64	chromosome 6 open reading frame 190
CD96						55.72	96.19	CD96 molecule
CECR1						448.29	1071.64	cat eye syndrome chromosome region, c...
CERKL						114.22	304.45	Ceramide kinase-like
CNTN1						287.34	790.98	Contactin 1
DHRS2						60.60	135.59	dehydrogenase/reductase (SDR family) ...
DTNB						24.66	33.93	Dystrobrevin, beta
EPHA7						40.44	97.08	EPH receptor A7
EPYC						38.75	144.34	epiphycan
FABP7						27.76	49.10	Fatty acid binding protein 7, brain
FAM123A						30.57	78.78	family with sequence similarity 123A
FAM19A1						36.95	99.38	family with sequence similarity 19 (c...
FAM9B						22.07	38.67	family with sequence similarity 9, me...
FMO3						50.40	154.19	flavin containing monooxygenase 3
GLRB						98.28	222.96	glycine receptor, beta
HMGCLL1						64.10	169.84	3-hydroxymethyl-3-methylglutaryl-Coen...
IL17A						20.43	35.79	interleukin 17A
LRP1B						41.83	80.45	Low density lipoprotein-related prote...
MAF						512.62	991.53	v-maf musculoaponeurotic fibrosarcoma...
MAOB						290.86	990.46	Monoamine oxidase B
MS4A1						65.09	105.17	Membrane-spanning 4-domains, subfamil...
MS4A2						49.89	149.70	membrane-spanning 4-domains, subfamil...
NAP5						31.46	77.04	Nck-associated protein 5
PAK3						282.22	968.44	P21 (CDKN1A)-activated kinase 3
PDE5A						306.09	874.74	phosphodiesterase 5A, cGMP-specific
PLD5						63.87	175.74	phospholipase D family, member 5
RAD54B						70.60	131.42	RAD54 homolog B (S. cerevisiae)
RNF180						206.28	463.86	ring finger protein 180
RSPO2						52.67	137.44	R-spondin 2 homolog (Xenopus laevis)
SCN7A						35.25	74.42	Sodium channel, voltage-gated, type V...
SIGLEC6						57.07	135.81	sialic acid binding Ig-like lectin 6
SPOCK3						899.67	2236.61	sparc/osteonectin, cwcv and kazal-lik...
SYNE1						104.74	222.65	spectrin repeat containing, nuclear e...
TLR10						35.21	72.70	toll-like receptor 10
ZNF533						76.41	177.56	Zinc finger protein 533

Figure 5. LCM *vs*. cell sorting. The transcriptome coverage of cells obtained by LCM is compared to that of sorted cells. Same amounts of RNA were used to perform the array analysis.

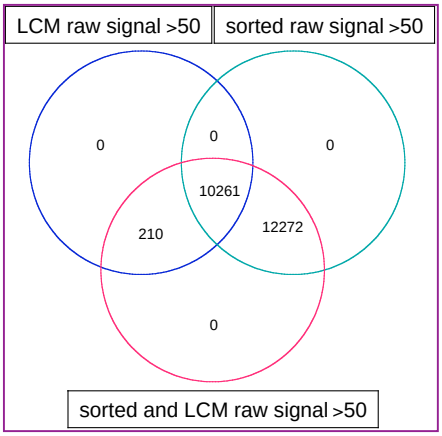


Figure 6. Secreted proteomes of cultured prostate and bladder stromal cells. The Venn diagram shows the overlap of the two proteomes, PS = prostate, BL = bladder. Altogether, 118 proteins were identified.

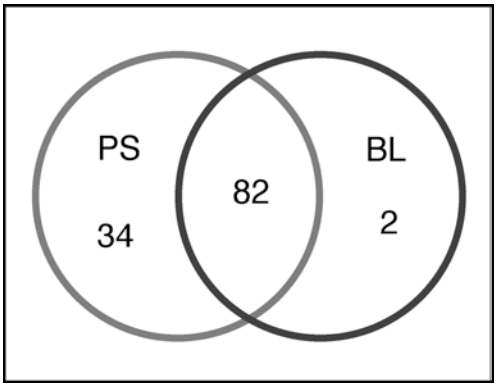


Figure 7. Gene Ontology subcellular categorization of the proteomes. Most of the identified secreted proteins, 76%, were categorized as extracellular or membrane-associated. NA = not available.

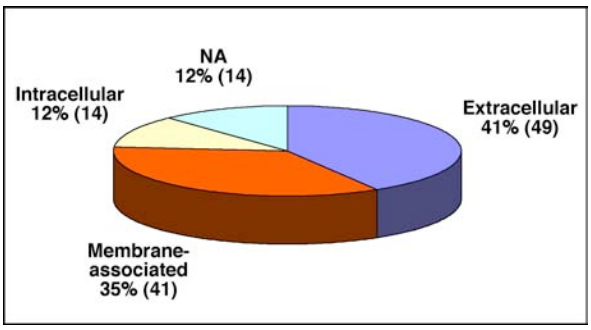


Figure 8. Gene Ontology molecular function distribution. Overall, a similar pattern is seen for the two proteomes but with protein binding and hydrolase activity being more prominent in the prostate group.

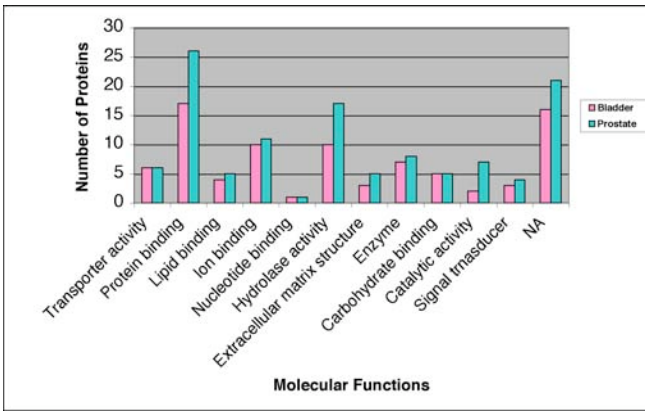


Figure 9. Gene Ontology biological process distribution. Prominent among the processes identified are physiology and communication.

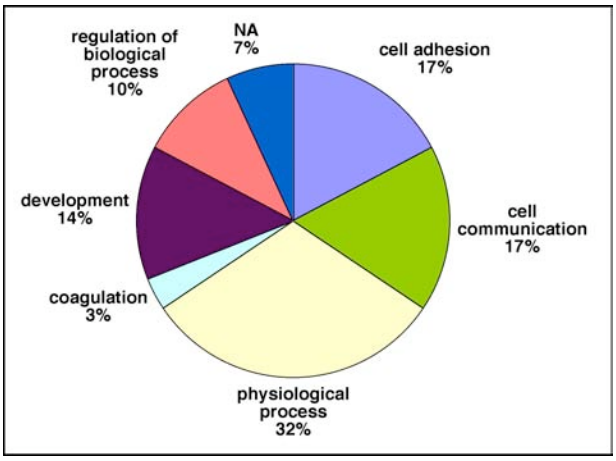


Figure 10. Western validation of differentially expressed proteins. Protein preparations of cultured cells were resolved by gel electrophoresis and probed by antibodies to gene products listed on the right. CD90 is the control for sample loading. The expression level as indicated by the band intensity correlated well with the PICA quantification method.

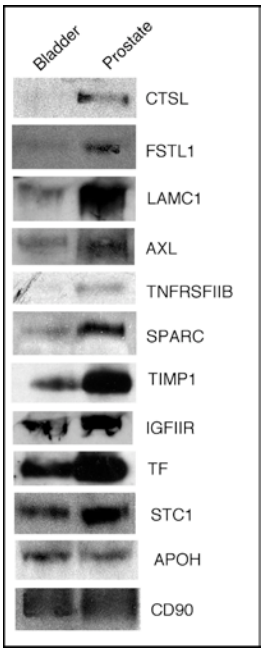


Figure 11. Expression of prostate stromal genes in cancer. NP and CP are matched non-cancer and cancer specimens processed into cDNA. Bone, liver, and lymph node metastasis specimens were obtained from end-stage diseases. CNTN1 is detectable in all NP samples, but lowered or barely detectable in CP as well as the metastases. MAOB and SPOCK3 showed similar expression in these same samples, though the differential expression was not as great. MAOB was detected in the lymph node metastasis but not detected in the bone and the liver metastasis. cDNA quantity of each sample used was monitored by ∝SMA (shown only for CP1/NP1).

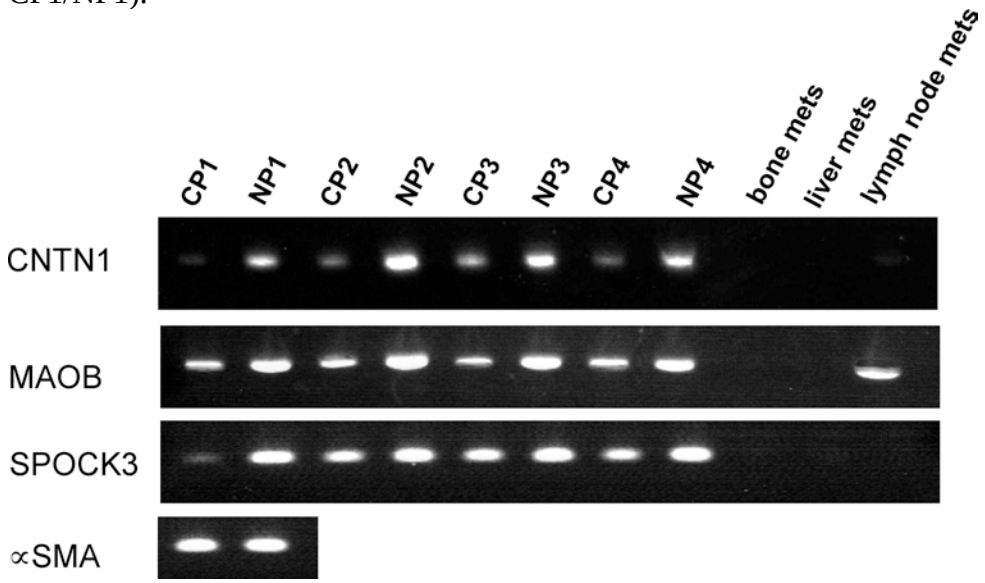


Table 1. Differentially expressed secreted proteins identified from the cultured prostate and bladder stromal cells. The protein IPI ID, ProteinProphet probability (PB) score, annotation, expression ratio, false discovery rate, peptide sequence (with putative glycosylation site underlined), GO biological process, GO molecular process, and GO cellular compartment information are tabulated. The list is sorted by FDR.

Protein	PB	Annotation	PS/BL	FDR	Peptides	Biological process	Molecular function	Cellular location
IPI00012887	1	Cathepsin L	4.9	0.001	YSVANDTGFVDIPKQEK YSVANDTGFVDIPK	physiological process	hydrolase activity	extracellular
IPI00029723	1	Follistatin-related protein 1	11.0	0.001	GS**NYS**EILDK FVEQ**NET**AI**NIT**TYPDQENNK VEQ**NET**AI**NIT**TYPDQENNK	NA	carbohydrate binding	extracellular
IPI00297160	1	Hypothetical protein DKFZp451K1918	3.7	0.001	AF**NST**LPTMAQMEK	cell adhesion	carbohydrate binding	membrane associated
IPI00029131	1	Neuroendocrine convertase 2	3.0	0.001	RGDLNI**NMT**SPMGTK RNPEAGVATTDLYG**NCT**LR NPEAGVATTDLYG**NCT**LR	physiological process	hydrolase activity	extracellular
IPI00015657	1	Pregnancy-specific beta-1-glycoprotein 5	9.9	0.001	ILILPSVTR**NET**GPYECEIR	physiological process	NA	extracellular
IPI00298281	1	Laminin gamma-1 chain	7.6	0.001	LLN**NLT**SIK V**NNT**LSSQISR TA**NDT**STEAYNLLLR	positive regulation of biological process	extracellular matrix structural	membrane associated
IPI00020986	1	Lumican	2.6	0.001	LHINHN**NLT**ESVGPLPK KLHINHN**NLT**ESVGPLPK	physiological process	protein binding	extracellular
IPI00339227	1	Splice Isoform 7 Of Fibronectin	7.5	0.002	DQCIVDDITYNV**NDT**FHK WTPL**NSS**TIIGYR HEEGHML**NCT**CFGQGR	cell adhesion	protein binding	extracellular
IPI00296992	0.99	AXL receptor tyrosine kinase, isoform 1	7.0	0.002	EESPFVGNPG**NIT**GAR	physiological process	transferase activity	membrane associated
IPI00298362	1	Tumor necrosis factor receptor superfamily member 11B	3.9	0.002	HIGHA**NLT**FEQLR KHT**NCS**VFGLLLTQK CPDGFFS**NET**SSK	development	protein binding	extracellular
IPI00014572	1	SPARC	7.2	0.002	VCSND**NKT**FDSSCHFFATK	development	ion binding	extracellular
IPI00009198	1	Tissue factor pathway inhibitor 2	4.0	0.002	DEGLCSA**NVT**R YFF**NLS**SMTCEK	coagulation	extracellular matrix structural	extracellular
IPI00328113	1	Fibrillin 1	7.4	0.002	TAIFAF**NIS**HVSNK	development	ion binding	extracellular
IPI00018305	0.99	Insulin-like growth factor binding protein 3	10.9	0.003	GLCV**NAS**AVSR	positive regulation of biological process	enzyme	extracellular
IPI00419941	1	PTK7 protein tyrosine kinase 7, isoform a	1.7	0.003	MHIFQ**NGS**LVIHDVAPEDSGR	physiological process	transferase activity	membrane associated
IPI00021081	1	Splice Isoform 1 Of Follistatin	2.2	0.003	SDEPVCASD**NAT**YASECAMK	development	protein binding	extracellular
IPI00032292	1	Metalloproteinase inhibitor 1	2.1	0.004	SH**NRS**EEFLIAGK FVGTPEV**NQT**TLYQR AKFVGTPEV**NQT**TLYQR VGTPEV**NQT**TLYQR	positive regulation of biological process	enzyme	extracellular
IPI00298828	1	Beta-2-glycoprotein I	0.2	0.006	VYKPSAG**NNS**LYR LG**NWS**AMPSCK	cell communication	carbohydrate binding	extracellular
IPI00291866	1	Plasma protease C1 inhibitor	0.5	0.006	DTFV**NAS**R NP**NAT**SSSSQDPESLQDR	physiological process	enzyme	extracellular
IPI00470937	1	Protein tyrosine phosphatase, receptor type, K	3.8	0.006	GPLANPIW**NVT**GFTGR	physiological process	hydrolase activity	membrane associated
IPI00289819	1	Cation-independent mannose-6-phosphate receptor	1.5	0.008	DAGVGFPEYQEED**NST**YNFR	physiological process	transferase activity	membrane associated
IPI00022810	1	Dipeptidyl-peptidase I	63.9	0.008	DV**NCS**VMGPQEK VTTYC**NET**MTGWVHDVLGR	cell communication	hydrolase activity	intracellular
IPI00169285	1	Hypothetical protein LOC196463	0.1	0.021	HPDAVAWA**NLT**NAIR	NA	NA	NA
IPI00023673	1	Galectin-3 binding protein	3.5	0.024	YKGL**NLT**EDTYKPR	cell	signal	extracellular

					DAGVVCT**NET**R AAIPSALDT**NSS**K ALGFE**NAT**QALGR	communication	transducer	
IPI00218019	0.99	Splice Isoform 1 Of Basigin	1.8	0.038	ILLTCSL**NDS**ATEVTGHR	cell communication	carbohydrate binding	membrane associated
IPI00016112	1	Melanoma associated gene	11.7	0.045	QGEHLS**NST**SAFSTR ILCDNAD**NIT**R LSTTECVDAGGESHA**NNT**K	cell communication	protein binding	NA
IPI00339223	1	Splice Isoform 3 Of Fibronectin	6.4	0.046	HEEGHML**NCT**CFGQGR RHEEGHML**NCT**CFGQGR DQCIVDDITYNV**NDT**FHK	cell adhesion	protein binding	extracellular
IPI00028931	0.98	Desmoglein 2	0.7	0.050	I**NAT**DADEPNTLNSK	cell adhesion	ion binding	membrane associated
IPI00003813	1	Nectin-like protein 2	0.7	0.050	VSLT**NVS**ISDEGR FQLL**NFS**SSELK DTAVEGEEIEV**NCT**AMASK	cell adhesion	protein binding	membrane associated

Meeting abstract 1

Comparative analysis of secreted proteins of human prostate and bladder stromal mesenchyme cells

Y. Goo[1,3], S. Ryu[1], L. Walashek[2,3], S. Shaffer[1], A. Liu[2,3], D. Goodlett[1,3]

[1]Medicinal Chemistry, University of Washington, Seattle, WA, USA. [2]Urology, University of Washington, Seattle, WA, USA, [3]Institute for Systems Biology, Seattle, WA, USA

Functional development of the prostate is governed by stromal mesenchyme induction and epithelial response. Stromal/epithelial signaling can be mediated through direct cell-cell contact and diffusible factors with their cell surface receptors. Given the importance of intercellular communication, it is possible that diseases such as benign prostatic hyperplasia and prostate cancer could arise from a change/loss of this communication. These inducers are likely secreted proteins or hormones differentially made by prostate and bladder stromal cells. In this study we identified secreted proteins that might be responsible for stromal/epithelial signaling and organ-specific induction of epithelial differentiation.

Prostate and bladder stromal cells in the third or fourth passage were placed in RPMI-1640 media without fetal bovine serum and cultured for 48 h. Cell-free media was prepared by centrifugation and filtration. Secreted proteins were identified by N-linked glycopeptide capture method using hydrazide chemistry and a LTQFT hybrid ion trap-Fourier Transform Ion Cyclotron Resonance mass spectrometer (FT-ICR-MS). Relative protein quantification was carried out using peak intensity areas and peptide spectral counts combined with a novel statistical package that omits the need for stable isotope labeling. This data produced a list of 71 and 51 secreted proteins with probability ≥ 0.9 from prostate and bladder stromal conditioned media, respectively. Of these stromal cell proteins, 75% (prostate) and 63% (bladder) had peptides containing an N-glycosylation sequence motif. The most differentially expressed proteins were ones functioning in cell-cell signaling, extracellular matrix interaction, processing and transport.

Our comparative stable isotope free proteomic analysis allowed 1) facile measurement of changes in protein expression using a modification of the N-linked glycopeptide capture method and 2) identification of a number of potential proteins for stromal/epithelial signaling and organ-specific induction. We believe these proteins possess potential as new diagnostic markers and therapeutic targets.

7th Siena Meeting: From Genome to Proteome. Siena, Italy Oct 2006, Poster presentation

Meeting abstract 2

Secreted glycoproteins of human prostate and bladder stromal mesenchyme cells

Young Ah Goo[1,2]; Soyoung Ryu[1]; Scott Shaffer[1]; Laura Walashek[1,2] ; Alvin Y. Liu[1,2]; David R. Goodlett[1,2]

[1]University of Washington, Seattle, WA; [2]Institute for Systems Biology, Seattle, WA

Functional development of the prostate is governed by stromal induction and epithelial response. Stromal/epithelial signaling can be mediated through direct cell-cell contact and diffusible factors with their cell surface receptors. These signaling factors are likely extra-cellular proteins, such as secreted proteins or cell surface membrane–associated proteins, made by stromal cells. N-linked glycosylation is a very common post-translational modification found in extra-cellular proteins and many clinical biomarkers, including prostate specific antigen (PSA), are glycoproteins. In this study we identified differently expressed secreted proteins from cell culture media of prostate and bladder stromal cells that might be responsible for stromal/epithelial signaling and differentiation.

Normal prostate and bladder tissues were obtained from non-cancerous part of the prostatectomy or cystoprostatectomy specimens. Tissue was digested, and stromal cells were separated by Percoll density gradient. Stromal cells in the third or fourth passage were placed in RPMI-1640 without FBS and cultured for 24 h. Culture media was collected and secreted proteins were identified by N-linked glyco-peptide capture method using hydrazide chemistry and an ion trap-Fourier Transform Ion Cyclotron Resonance mass spectrometer (FT-ICR-MS). Relative protein quantification between prostate and bladder was carried out using peak intensity areas and peptide spectral counts combined with a novel statistical package that omits the need for stable isotope labeling.

This data produced a list of 120 and 88 secreted glycoproteins with protein probability greater than 0.9 from prostate and bladder stromal conditioned media, respectively. The most differentially expressed proteins in prostate stromal cells were those functioning in cell-cell signaling, extra-cellular matrix interaction, processing, and transport. One interesting observation was increased expression of signal transducers like AXL receptor tyrosine kinase, and protein tyrosine kinase 7, which have been implicated in tumor progression. The differential expression of the proteins was confirmed by Western blot analysis.

Our comparative stable isotope-free proteomic analysis allowed facile measurement of changes in protein expression using a modification of the N-linked glyco-peptide capture method, and identification of a number of secreted proteins which may be involved in stromal/epithelial signaling and organ-specific differentiation.

USHUPO 3rd Annual Conference. Seattle, USA Mar 2007, Poster presentation

Meeting abstract 3

Identification of human prostate and bladder stromal factors by quantitative transcriptome and proteomics analysis

Goo, YA., Liu, AY., Goodlett, DR.

Functional development of the prostate is governed by stromal induction and epithelial response. Stromal/epithelial signaling can be mediated through direct cell-cell contact and diffusible factors with their cell surface receptors. Given the importance of intercellular communication, it is possible that diseases such as cancer could arise from a loss of this communication. One approach to gain a molecular understanding of epithelial/stromal interaction is to identify the organ-specific stromal signaling factors. We proposed to do this through a comparative analysis between prostate and bladder stromal cells. Stromal signaling factors are made when the stromal cells are grown in culture since epithelial induction can be achieved with stromal cell conditioned media. As such, quantitative proteomic analysis was carried out to identify differentially expressed secreted proteins found in the culture media of prostate and bladder stromal cells.

For the prostate/bladder transcriptome analysis, we isolated stromal cell populations by the use of specific CD markers: CD49a for prostate and CD13 for bladder with magnetic cell sorting and laser-capture microdissection. The Affymetrix microarray platform was used to determine the cell type-specific transcriptomes. We have identified more than 100 genes that are up-regulated in the CD49a prostate cells (3-fold or more). The most differentially expressed gene was SPARC/osteonectin proteoglycan 3 (SPOCK3). SPOCK3 interferes with tumor invasion by inhibiting matrix metallopeptidases. More than 80 genes were also up-regulated in the CD13 bladder cells, the most being transient receptor potential cation channel (TRPA1). TRPA1 expression was reported to be down-regulated in mesenchymal tumor cells. Secreted proteins were identified by N-linked glycopeptide-capture method followed by an ion trap-fourier transform ion cyclotron resonance mass spectrometer (FT-ICR-MS). Relative protein quantification between prostate and bladder was carried out using peak intensity areas, combined with a novel statistical package. This experiment produced a list of 120 and 98 secreted glycoproteins from prostate and bladder stromal conditioned media, respectively. The most differentially expressed proteins in prostate stromal cells were those functioning in cell-cell signaling, extra-cellular matrix interaction, processing, and transport. One interesting observation was increased expression of cathepsin L precursor (CTSL), which has been implicated in tumor progression in lung cancer. Western blot analysis was performed to validate their expression.

Our comparative transcriptome and stable isotope-free proteomic analysis allowed facile measurement of differences in gene and protein expression. A number of genes and secreted proteins, which may be involved in stromal/epithelial signaling and organ-specific differentiation, were identified.

IMPACT: This project could potentially have a great impact on prostate cancer research by providing biomarker targets for diagnosis and treatment. Stromal/epithelial interaction is evident in the differentiative development of a number of organs. As such, diseases may arise due to defects in this process. Secreted proteins often play a major role in intercellular communication, and changes in their expression in cancer may implicate a loss or malfunctioning in communication via these signaling molecules. These factors can be targeted in therapeutic regimens.

CDMRP PCRP 2007 IMPaCT Meeting. Atlanta, USA Sep 2007, Poster presentation

Meeting abstract 4

Identification of human prostate and bladder stromal cell signaling factors by quantitative proteomic and transcriptome analysis

Young Ah Goo, Alvin Y. Liu, David R. Goodlett
University of Washington, Seattle, WA. USA

Functional development of the prostate is governed by stromal induction and epithelial response. Stromal/epithelial signaling can be mediated through direct cell-cell contact and diffusible factors with their cell surface receptors. Given the importance of intercellular communication, it is possible that diseases such as cancer could arise from a loss of this communication. One approach to gain a molecular understanding of epithelial/stromal interaction is to identify the organ-specific stromal signaling factors. In this study, comparative proteomic and transcriptome analysis were carried out to identify differentially expressed secreted proteins and genes from prostate and bladder stromal cells.

Secreted proteins were identified by *N*-linked glycopeptide-capture method followed by an ion trap-fourier transform ion cyclotron resonance mass spectrometer (FT-ICR-MS). Relative protein quantification was carried out using a novel label-free method, PICA (Peptide Ion Current Area). A list of 116 prostate and 84 bladder secreted glycoproteins was identified. The identified prostate stromal proteins include cathepsin L, neuroendocrine convertase, tumor necrosis factor receptor, and others that are known to be involved in signal transduction, extracellular matrix interaction, and differentiation.

For the prostate/bladder transcriptome analysis, cells were isolated by the specific CD markers: CD49a for prostate and CD13 for bladder with magnetic cell sorting. The Affymetrix microarray platform was used to determine the cell type-specific transcriptomes. In a comparative analysis, 91 bladder and 288 prostate genes were identified. Further subtractive analysis resulted in a list of 50 prostate stromal cell-specific genes. Interesting genes were CNTN1, SPOCK3, and MAOB, which are known to be involved in cell interactions and organogenesis. Expression analysis showed that these genes are down-regulated in cancer, suggesting their function is required in normal development.

Our comparative proteomic and transcriptome analysis allowed facile measurement of differences in protein and gene expression. A number of secreted proteins and genes, which may be involved in stromal/epithelial signaling and organ-specific differentiation, were identified.

HUPO 6th Annul World Congress. Seoul, Korea Oct 2007, Poster presentation

Identification of secreted glycoproteins of human prostate and bladder stromal cells by comparative quantitative proteomics

Journal:	*PROTEOMICS*
Manuscript ID:	draft
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Goo, Young Ah; University of Washington, Medicinal Chemistry Liu, Alvin; University of Washington Ryu, Soyoung; University of Washington Shaffer, Scott Malmstroem, Lars; University of Washington Page, Laura Nguyen, Liem Doneanu, Catalin Goodlett, David
Key Words:	bladder, Prostate, Secreted protein, glycoprotein, stromal cells

Identification of secreted glycoproteins of human prostate and bladder stromal cells by comparative quantitative proteomics

Young Ah Goo[1,2,3], Alvin Y. Liu[2,3], Soyoung Ryu[1], Scott A. Shaffer[1], Lars Malmstroem[1], Laura Page[2] , Liem T. Nguyen[1], Catalin E. Doneanu[4], and David R. Goodlett[1,3*]

[1]Medicinal Chemistry (Box 357610), [2]Urology (Box 356510), University of Washington, Seattle, WA 98195, [3]Institute for Systems Biology, 1441 N. 34th St. Seattle, WA, 98103, [4]Waters Corporation, 34 Maple St. Milford, MA, 01757.

* Corresponding Author:

University of Washington

Dept. of Medicinal Chemistry

Box 357610

Seattle, WA 98195-7610, USA

Tel: 206-616-4586, Fax: 206-685-3252

goodlett@u.washington.edu

Abbreviations: extracellular matrix (ECM), proenkephalin (PENK), laser capture microdissection (LCM), prostate cancer (CP), normal prostate (NP), peptide ion current area (PICA)

Key words: bladder, glycoprotein, prostate, secreted protein, stromal cells

ABSTRACT

Functional development of the prostate is governed by stromal mesenchyme induction and epithelial response. Stromal/epithelial signaling can be mediated through direct cell-cell contact and diffusible factors and their cell surface receptors. These inducers are likely secreted or membrane-associated extracellular proteins. Given the importance of intercellular communication, it is possible that diseases like cancer could arise from a loss of this communication. One approach to gain a molecular understanding of epithelial/stromal interaction is to identify, as a first step, the organ-specific stromal signaling factors. In this study, we identified differentially expressed secreted proteins from prostate and bladder stromal mesenchyme cells by using cells cultured from tissue specimens. Differences in protein abundance were quantified from calculated peptide ion current area (PICA) via a software package that omits the need for stable isotope labeling. This analysis produced a list of 116 prostate and 84 bladder secreted glycoproteins with probability scores ≥ 0.9. The identified prostate stromal proteins include cathepsin L, follistatin-related protein, neuroendocrine convertase, tumor necrosis factor receptor, and others that are known to be involved in signal transduction, extracellular matrix interaction, differentiation and transport. Our comparative stable isotope-free proteomic analysis allowed for a facile measurement of protein quantity using the glycopeptide-capture method.

INTRODUCTION

The prostate develops from the urogenital sinus, and this development is governed by stromal induction and epithelial response. In addition to interaction between epithelium and stromal mesenchyme, morphogenesis and functional cytodifferentiation are dependent on interactions between epithelium and basement membrane, and the extracellular matrix (ECM).[1] Homeostasis of the prostate is maintained by reciprocal signaling between stromal and epithelial cells. There is also evidence that stromal-epithelial interaction is involved in the development of the gut[2] and the kidney.[3] In a comparative cDNA microarray analysis of cultured prostate and bladder stromal cells, proenkephalin (PENK) was shown to have the highest fold difference in expression.[4] Prostate-specific expression of PENK was verified by RT-PCR analysis of stromal cells obtained by laser capture microdissection (LCM) and database analysis of prostate cell transcriptomes. RT-PCR analysis of matched prostate cancer (CP) and normal prostate (NP) cDNA showed undetectable or decreased PENK expression in CP. Given the importance of intercellular communication, it is possible that diseases such as benign prostatic hyperplasia and prostate cancer could arise from a loss of or defect in this communication. Investigation into how the normal stromal/epithelial interaction is affected in cancer may lead to a better understanding of the cancer process.

It is hoped that a molecular understanding of prostate carcinogenesis will in turn lead to effective therapies, and more importantly, preventive measures. One of the keys lies in the identification of stromal cell factors that mediate stromal/epithelial interaction, expression of which is abnormal in cancer. These signaling factors are most likely extracellular proteins, such as secreted or membrane–associated proteins. *N*-linked glycosylation is a common post-translational modification found in extracellular proteins, and the analytical strategy used in this

work is targeting this class of proteins. Cultured stromal cells were used to provide enough material for proteomics analysis. It has been shown that epithelial induction can be achieved with stromal cells in co-culture system.[5,6] Therefore, these signaling molecules are still being synthesized when in culture. There are also several reported studies that showed media from cultured stromal cells could stimulate migration of prostate epithelial cells and proliferation of breast epithelial cells. [7,8] In our unpublished work, a pluripotent embryocarcinoma cell line, NCCIT, was used as an in vitro model to demonstrate the inductive property of prostate stromal cells. Diffusible factors (i.e., present in culture media) made by prostate stromal cells could induce morphologic, cell surface antigen and gene expression changes including loss of stem cell markers in the treated NCCIT cells (Pascal, L. *et al*., submitted for publication). This cumulative evidence suggests that stromal signaling molecules can be identified from an analysis of culture media.

Conventional quantitative methods of measuring changes in protein expression generally utilize comparison of protein "spot" intensities on 2DE gels.[9] While the resolving power of 2DE-MS (mass spectrometry) is arguably its best feature, it does suffer from loading capacity problems which affect ultimate achievable sensitivity, and its inability to directly analyze membrane proteins. Shotgun proteomic methods circumvent these problems by direct MS analysis of complex protein mixtures.[10] The isotope coded affinity tag (ICAT) based shotgun proteomic strategy for protein expression profiling is predicated on distinguishing two protein populations that are labeled with two different stable isotope "mass" tags.[11] The ICAT method is useful for pair-wise comparisons, but is unwieldy and expensive for analysis of large numbers of samples. The newer iTRAQ method (Applied Biosystems, Foster City, CA) attempts to overcome these shortcomings, but even it can only provide up to 4-, 8-wise comparisons.

Statistical-based "label-free" quantification methods provide for analysis of large numbers of samples that circumvents pair-wise or higher experimental design limitations. As such, an enhanced stable isotope-free quantitative proteomic analysis was carried out to identify differentially expressed secreted proteins in the culture media of prostate and bladder stromal cells. This comparative analysis was expected to identify many, if not all, differentially expressed proteins that could play a role in organ-specific intercellular communication.

MATERIALS AND METHODS

Prostate and bladder tissue preparation and cell culture

Human tissue acquisition was carried out under UW IRB approval. Prostate specimens were obtained from patients undergoing radical prostatectomy for their cancer treatment. Bladder specimens were obtained from patients undergoing cystoprostatectomy for their cancer treatment. Normal prostate and bladder tissues were harvested from non-cancerous part of the resected organs. Cell cultures were started either by placing cut tissue pieces on tissue culture plates or by plating single cells prepared by tissue digestion with collagenase as described previously.[4] Briefly, minced tissue was placed in RPMI-1640 media supplemented with 5% fetal bovine serum (FBS), gentamicin, and 10^{-8} M dihydrotestosterone (DHT), and digested with type I collagenase (Invitrogen, Carlsbad, CA) at room temperature overnight with gentle stirring. The digested tissue was passed through Falcon 70µm filter (Becton-Dickinson, Franklin Lakes, NJ) and aspirated with 18-gauge needle. The cell suspension was partitioned from epithelial cells by centrifugation in a discontinuous Percoll density gradient. Prostate or bladder stromal cells taken from the Percoll gradients were cultured in RPMI-1640 supplemented with 10% FBS and DHT. Cells were trypsinized and serially passaged. Light microscopy was used to check cell

morphology. Cells were monitored for expression of smooth muscle actin (αSMA) and non-expression of epithelial cell-specific glycoprotein (EGP) by RT-PCR (Figure 1). Primer pairs (and expected product size) used were: αSMA, 5' GCCTCTGGACGCACAACTGGCATCG and 3' GTTTGCTGATCCACATCTGCTGGAAGG (650 bp); EGP, 5' TGGAGGTGCCG TTGCACTGCTT and 3' CGACTTTTGCCGCAGCTCAGGA (290 bp). For positive EGP expression, the prostate cancer cell line, LNCaP, was used. Percoll gradients were used additionally to remove any contaminant epithelial cell types at serial passages.

Glycopeptide-capture proteomics by mass spectrometry

Prostate and bladder stromal cells (10^7 cells) in the third or fourth passage were placed in RPMI-1640 without FBS and cultured for 24 h. This duration was based on our previous experiments that showed no induction of serum deficiency shock proteins within this time period (unpublished data). The serum-free media treatment was called for because the FBS supplement would overwhelm proteomic analysis with bovine albumin and other abundant serum proteins. The media was made cell-free by centrifugation and filtration. Proteins in the media were concentrated using Centricon Plus-20 (NMWL: 5000, Millipore, Billerica, MA). Secreted proteins were analyzed using the *N*-linked glycoprotein-capture method.[12] Briefly, 1 mg protein from each sample was exchanged into coupling buffer (100 mM NaAc and 150 mM NaCl, pH 5.5) using a desalting column (Bio-Rad, Hercules, CA), and oxidized by adding 15 mM sodium periodate at room temperature for 1 h. After removal of the oxidant with a desalting column, the sample was conjugated to hydrazide resin (Bio-Rad) at room temperature for 10–24 h. Non-glycosylated proteins were removed by washing the resin three times with 1 ml of 8 M urea, 0.4 M NH_4HCO_3, pH 8.3. After the last wash, proteins were reduced in 8 mM Tris-(2-carboxyethyl)

phosphine at room temperature for 30 min and alkylated in 10 mM iodoacetamide for 30 min. The resin was washed twice with 1 ml 0.1 M NH_4HCO_3, trypsin was added at 1 mg/200 mg protein for digestion at 37°C overnight. The trypsin-cleaved peptides were removed, and the resin was washed three times successively each in the following solutions: 1.5 M NaCl, 80% acetonitrile (ACN), 100% methanol, water, and 0.1 M NH_4HCO_3. *N*-Linked glycopeptides were released from the resin by 0.3 µl peptide-N-glycosidase F (PNGase F, 500 units/µl, New England Biolabs, Beverly, MA) at 37°C overnight. Released peptides were dried and resuspended in 50% ACN, 0.1 % formic acid for MS analysis. Four experimental replicates each for prostate and bladder were analyzed by LCMS using a Michrom Paradigm MS4B HPLC system (Michrome Bioresources, CA) that was coupled via electrospray ionization (ESI) on-line to a linear ion trap (LTQ) Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (LTQ-FT, ThermoFinnigan, CA).

Identification and analysis of glycoproteins

Glycopeptides were fractionated by online C18 reverse phase cation-exchange chromatography into four fractions, and each fraction was analyzed. Tandem mass spectral RAW (ThermoFinnigan) files were converted to mzXML format using the program ReAdW[13] and subsequently directly exported to .dta files without further processing using the MzXML2Search program (http://sashimi.sourceforge.net/viewvc/sashimi/trunk/trans_proteomic_pipeline/src/Parsers/MzXML2Search/). Acquired MS/MS spectra were searched for sequence matches against the International Protein Index (IPI) human protein database (version 3.01) using SEQUEST. The following modifications were set as search parameters: parent ion and fragment mass tolerance at 1.2, trypsin digestion (cleavage after K or R except if followed by

a P), 1 allowed missed cleavage site, carboxymethylated cysteines, oxidized methionines, and conversion of Asn (N) to Asp (D) at N-glycosites by PNGase F. PeptideProphet and ProteinProphet, which compute a probability likelihood of each identification being correct, were used for statistical analysis of search results.[14] A PeptideProphet probability score of ≥ 0.5 was chosen as a filter which corresponds to an error rate of ≤ 3%. For proteins, a ProteinProphet probability score of ≥ 0.9 was applied which corresponds to less than a 0.02% error rate. For proteins identified by one unique peptide sequence, they were included in the study when multiple observations (≥ 2) of the same peptide are seen. To reduce the false positive rate further, peptides were filtered for the N-linked glycosylation motif (N-X-S/T where X is any amino acid except proline).

Isotope–free labeling quantitative analysis of glycoproteins

Samples were analyzed in quadruplicate by LC-MS on the LTQ-FT. Reproducibility of eluting peptide ion map patterns (i.e. *m/z* vs. time) and relative peptide ion intensity values between runs was tested using The Dragon Visualization tool.[15] Ion map alignment patterns of the top 4,000 peptides among the replicates indicated satisfactory reproducibility (data not shown). Expression levels of proteins were estimated using PICA (peptide ion current area, Ryu, S., *et al*., submitted for publication) with a modification of ion intensity areas as previously described.[16-18] Peptide ion intensities were normalized using total ion intensity following background noise subtraction, and retention time shift between runs was adjusted to align identical peptides from separate analyses. All peptides identified for a given protein were collected, and theoretical *m*/*z* of the first three isotopic peaks of the peptide were calculated. These three isotopic peaks were smoothed over time using the Savitzky-Golay filter and extracted for further analysis only when

they persisted in both accurate *m/z* (± 0.015) and respective retention time (initially, within ± 3 minutes). Only isotopic peaks with three or more scans to reconstruct the ion chromatogram were extracted in the study. Protein expression was estimated by summing the ion current areas of the corresponding peptides. The protein ratio between bladder and prostate was derived by dividing an average protein expression value of bladder by that of prostate. Differentially expressed proteins were identified by two-sample *t*-test (with unequal variance) and false discovery rate (FDR), which corrected for multiple testing error. Proteins with FDR ≤ 0.05 were considered to be significant for differential expression and subjected to further analysis.

Western blot analysis

Differentially expressed proteins identified via the label-free quantification method were validated by Western blot analysis. Five to 10 µg of bladder or prostate protein was resolved on 4-12% NuPAGE® Bis-Tris gel (Invitrogen) and transferred to PVDF or nitrocellulose membrane for incubation with primary antibodies, followed by washes and incubation with HRP-conjugated secondary antibodies (Amersham Biosciences, Piscataway, NJ). Reactivity was visualized by enhanced chemiluminescence (Amersham Biosciences). Protein molecular weight was estimated using the Dual color protein standard (Bio-Rad Laboratories). For the targeted proteins, if the first antibody used failed, then one other antibody from a different vendor was tested. The commercial sources of antibodies for the following proteins were: FSTL1 (follistatin related protein), LAMC1 (laminin γ1), AXL (receptor tyrosine kinase), IGF2R (cation-independent mannose-6-phosphate receptor), and STC1 (stanniocalcin) - Santa Cruz Biotechnology (Santa Cruz, CA); TIMP1 (metalloproteinase inhibitor 1), APOH (β-2-

glycoprotein I) - Chemicon (Temecula, CA); TNFRSF11B (tumor necrosis factor receptor superfamily member 11B) - Aviva Systems Biology (San Diego, CA); SPARC (secreted acidic cysteine rich glycoprotein) - Abcam (Cambridge, MA); TF (serotransferrin) - Biovender (Chandler, NC); CTSL (cathepsin L) and CD90 - BD PharMingen (San Diego, CA). The CD90 antibody was used to control for equal gel loading of protein. CD90 is a GPI-anchored protein found released into the culture media by stromal cells, and the expression of CD90 in cultured bladder and prostate stromal cells has been previously reported.[19]

RESULTS

Secreted proteins of human prostate and bladder stromal cells

A total of 116 glycoproteins were identified in the media of prostate stromal cell cultures and, of these, 34 were identified in the prostate but not the bladder. A total of 84 glycoproteins were identified in the media of bladder stromal cell culture and 2 of these were identified in the bladder but not the prostate. The two secreted proteomes thus shared expression of 82 proteins (Figure 2). Caution is needed in these types of comparisons because proteins identified in one sample only may be due to under-sampling by data-dependent ion selection,[20] data filtering, or peptides that failed to generate the necessary 3 isotopic peaks for inclusion for quantification. Western blotting as described below was used for validation. The complete list of identified proteins is provided in the data supplement.

Identified glycoproteins were annotated with Gene Ontology (GO)[21], which assigns putative cellular compartmentalization and molecular functions. Not surprisingly, >75% of the proteins identified by the glycopeptide-capture method were annotated as secreted or membrane-associated molecules (Figure 3). Protein- and ion-binding functions, hydrolase activity (catalytic

hydrolysis of C-O, C-N, C-C, phosphoric anhydride bonds, etc.) were the most common ascribed to these secreted proteins (Figure 4). Both bladder and prostate proteins exhibited a similar categorization in their GO functions. The binding, signal transduction, and transport functions could all be characteristic for those involved in intercellular communication.

Differences between proteins secreted by prostate and bladder stromal cells

A total of 29 proteins with FDR $\leq$ 0.05 were considered to be statistically significant for differential expression between prostate and bladder (Table 1). Only three of these 29 proteins were annotated in GO as either intracellular or unknown, all others were as secreted or membrane-associated cell surface proteins. When queried for GO biological processes, these proteins were found to be involved predominantly in physiology, cell adhesion, communication, and development (Figure 5). Of these differentially expressed proteins, quantitative proteomic analysis found that 24 were over-expressed in the prostate and 5 in the bladder. Their differential expression was validated by Western blot analysis (Figure 6). The limitation was that for some proteins, either no antibody was available or both antibodies failed to detect the targets. Detection failure could be due to many reasons, among which could be antibody quality, protein structure, amount of accessible antigen, etc. Nevertheless, for those that were detected, their MS derived differential expression between prostate and bladder was verified. Two prostate proteins, TF and STC1, with FDR >0.05 were also included for Western. STC1 was detected only in the prostate by MS but its expression was detected in both prostate and bladder by Western showing an increased level in the prostate.

Pathway analysis of the secreted proteins and implication in cell-cell signaling

All of the secreted proteins were analyzed for their pathway involvement by Teranode pathway analysis software[22] through the KEGG *Homo sapiens* database. Table 2 shows the number of pathways identified by two or more secreted proteins found in this study. Identification of such pathways suggested that these pathways might be active in stromal cell biology. The ECM-receptor interaction, complement and coagulation cascades, focal adhesion and cell adhesion were all mapped with the most number of secreted proteins. For example, in ECM-receptor interaction, the upregulation of FN and LAMC protein family in prostate stromal cells relative to bladder stromal cells was displayed (Figure 7). In this diagram, grey nodes indicate the component proteins of this pathway, and the 6 identified proteins are given different colors to indicate expression levels in which red is to signify decreased, green increased, and yellow as the midpoint. Useful information included for each identified protein is the IPI ID, ProteinProphet probability score, annotation, tissue type expression, prostate/bladder expression ratio, and associated FDR.

DISCUSSION

In this study, we identified a number of secreted stromal proteins that are candidate signaling molecules in tissue homeostasis. Of the prostate proteins, CTSL was the most differentially expressed protein in prostate stromal cells. In the literature, this lysosomal cysteine protease is reported to be synthesized in large amounts and secreted by tumor cells in culture. Tumor CTSL activity was proposed to facilitate invasion and metastasis by its degradation of ECM components.[23-25] An elevated CTSL level has been observed in primary cell cultures from

tumors.[26] Prostate stromal CTSL may well be one of the factors in tumor cell promotion. FSTL1 modulates the action of growth factors on cell proliferation and differentiation. It has structural similarity to the bone morphogenetic protein antagonist follistatin, as well as to the ECM modifying BM-40/SPARC/osteonectin. In mouse development, FSTL1 is strongly expressed in the mesenchyme, but not the epithelium.[27] PCSK2 (neuroendocrine convertase 2) releases hormones and neuropeptides from their precursors. PSG5 (pregnancy-specific β-1-glycoprotein), TNFRSF11B, FBN1 (fibrillin), IGFBP3 (insulin-like growth factor binding protein), and TIMP1 have been reported to be expressed in carcinoma-associated fibroblasts and normal gland-associated fibroblasts.[28,29] Membrane bound receptors including AXL, PTK7 (protein tyrosine kinase), PTPRK (protein tyrosine phosphatase receptor type K), and IGF2R were previously reported to be upregulated in prostate stromal cells. AXL appears to mediate cell growth and survival and to be involved in prostate cancer progression.[30] PTK7 functions as a cell adhesion molecule and has been implicated in gastric cancer as a candidate oncogene.[31] PTPRK regulates growth hormone signaling in breast cancer cells by interacting with phosphatase receptors.[32] IGF2R transports phosphorylated lysosomal enzymes from the Golgi complex and cell surface to lysosomes. It also functions as an IGF2 receptor.[33] CTSC (Dipeptidyl-peptidase I) is a thiol protease that activates serine proteases such as elastase and cathepsin G. Dipeptidyl peptidases were implicated as inhibitors of prostate cancer by their blocking of basic fibroblast growth factor.[34]

For the bladder proteins, APOH (β-2-glycoprotein I, apolipoprotein H) appears to prevent activation of the intrinsic blood coagulation cascade by binding to phospholipids on the surface of damaged cells.[35] SERPING1 (plasma protease C1 inhibitor) was reported to regulate physiological pathways including complement activation, blood coagulation, fibrinolysis and the

generation of kinins.[36] Defects in SERPING1 are the cause of hereditary angioneurotic edema. DSG2 (desmoglein) is involved in the interaction of plaque proteins and intermediate filaments in cell-cell adhesion.[37] CADM1 (nectin-like protein 2) is a cell adhesion molecule.

The enhanced label-free quantification method we employed could effectively identify differential expression between samples. This provides a major advance for when analysis of tens of clinical samples is needed, particularly as data on each sample is acquired independently of all others, and changes in proteins expression are calculated between any pair of samples. The finding that the majority of the differentially expressed secreted proteins were proteases, kinases, and growth factors involved in protein binding, transport and/or signal transductions suggests their possible involvement in stromal and epithelial communication, organ-specific development, and cell differentiation. Stromal secretion of cytokines, growth factors, and proteases is essential for epithelial differentiation and morphogenesis. Some of these factors are also involved in reciprocal stromal/epithelial interaction in prostate cancer.[38] Thus, stromal/epithelial signaling may be accomplished through transmission via activation of the ECM-receptor and focal adhesion pathways. With their identification, one can begin to study their biological function and we are developing an in vitro assay system to test the functional property of these identified proteins.

REFERENCES

1. Bissell, M. J. and Ram, T. G., Regulation of functional cytodifferentiation and histogenesis in mammary epithelial cells: role of the extracellular matrix. *Environ Health Perspect* 1989, 80, 61-70.

2. Kedinger, M., Lefebvre, O., Duluc, I., Freund, J. N. and Simon-Assmann, P., Cellular and molecular partners involved in gut morphogenesis and differentiation. *Philos Trans R Soc Lond B Biol Sci* 1998, 353, 847-856.

3. Saxen, L., What is needed for kidney differentiation and how do we find it? *Int J Dev Biol* 1999, 43, 377-380.

4. Goo, Y. A., Goodlett, D. R., Pascal, L. E., Worthington, K. D.*, et al.*, Stromal mesenchyme cell genes of the human prostate and bladder. *BMC Urol* 2005, 5, 17

5. Daly-Burns, B., Alam, T. N., Mackay, A., Clark, J.*, et al.*, A conditionally immortalized cell line model for the study of human prostatic epithelial cell differentiation. *Differentiation* 2007, 75, 35-48.

6. Lang, S. H., Smith, J., Hyde, C., Macintosh, C.*, et al.*, Differentiation of prostate epithelial cell cultures by matrigel/ stromal cell glandular reconstruction. *In Vitro Cell Dev Biol Anim* 2006, 42, 273-280.

7. You, X., Yu, H. M., Cohen-Gould, L., Cao, B.*, et al.*, Regulation of migration of primary prostate epithelial cells by secreted factors from prostate stromal cells. *Exp Cell Res* 2003, 288, 246-256.

8. Dong-Le Bourhis, X., Berthois, Y., Millot, G., Degeorges, A., *et al.*, Effect of stromal and epithelial cells derived from normal and tumorous breast tissue on the proliferation of human

breast cancer cell lines in co-culture. *Int J Cancer* 1997, 71, 42-48.

9. Yan, J. X., Sanchez, J. C., Tonella, L., Williams, K. L. and Hochstrasser, D. F., Studies of quantitative analysis of protein expression in Saccharomyces cerevisiae. *Electrophoresis* 1999, 20, 738-742.

10. Goodlett, D. R., Keller, A., Watts, J. D., Newitt, R., *et al.*, Differential stable isotope labeling of peptides for quantitation and de novo sequence derivation. *Rapid Commun Mass Spectrom* 2001, 15, 1214-1221.

11. Gygi, S. P., Rist, B., Gerber, S. A., Turecek, F., *et al.*, Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. *Nat Biotechnol* 1999, 17, 994-999.

12. Zhang, H., Li, X. J., Martin, D. B. and Aebersold, R., Identification and quantification of N-linked glycoproteins using hydrazide chemistry, stable isotope labeling and mass spectrometry. *Nat Biotechnol* 2003, 21, 660-666.

13. Pedrioli,. P. G., Eng, J. K., Hubley, R., Vogelzang, M., *et al.*, A common open representation of mass spectrometry data and its application to proteomics research. *Nat Biotechnol* 2004, 22, 1459-1466.

14. Goo, Y. A., Yi, E. C., Baliga, N. S., Tao, W. A., *et al.*, Proteomic analysis of an extreme halophilic archaeon, Halobacterium sp. NRC-1. *Mol Cell Proteomics* 2003, 2, 506-524.

15. Radulovic, D., Jelveh, S., Ryu, S., Hamilton, T. G., *et al.*, Informatics platform for global proteomic profiling and biomarker discovery using liquid chromatography-tandem mass spectrometry. *Mol Cell Proteomics* 2004, 3, 984-997.

16. Colinge, J., Chiappe, D., Lagache, S., Moniatte, M. and Bougueleret, L., Differential proteomics via probabilistic peptide identification scores. *Anal Chem* 2005, 77, 596-606.

17. Liu, H., Sadygov, R. G. and Yates, J. R., 3rd, A model for random sampling and

estimation of relative protein abundance in shotgun proteomics. *Anal Chem* 2004, 76, 4193-4201.

18. Wiener, M. C., Sachs, J. R., Deyanova, E. G. and Yates, N. A., Differential mass spectrometry: a label-free LC-MS method for finding significant differences in complex peptide and protein mixtures. *Anal Chem* 2004, 76, 6085-6096.

19. Liu, A. Y., LaTray, L. and van Den Engh, G., Changes in cell surface molecules associated with in vitro culture of prostatic stromal cells. *Prostate* 2000, 44, 303-312.

20. Yi, E. C., Marelli, M., Lee, H., Purvine, S. O., *et al.*, Approaching complete peroxisome characterization by gas-phase fractionation. *Electrophoresis* 2002, 23, 3205-3216.

21. Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., *et al.*, Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet* 2000, 25, 25-29.

22. Goo, Y., Li, Z., Pajkovic, N., Shaffer, S., *et al.*, Systematic investigation of lycopene effects in LNCaP cells by use of novel large-scale proteomic analysis software. *Proteomics Clin Appli* 2007 1, 513-523.

23. Chauhan, S. S., Goldstein, L. J. and Gottesman, M. M., Expression of cathepsin L in human tumors. *Cancer Res* 1991, 51, 1478-1481.

24. Staack, A., Tolic, D., Kristiansen, G., Schnorr, D., *et al.*, Expression of cathepsins B, H, and L and their inhibitors as markers of transitional cell carcinoma of the bladder. *Urology* 2004, 63, 1089-1094.

25. Shuja, S., Sheahan, K. and Murnane, M. J., Cysteine endopeptidase activity levels in normal human tissues, colorectal adenomas and carcinomas. *Int J Cancer* 1991, 49, 341-346.

26. Friedrich, B., Jung, K., Lein, M., Turk, I., *et al.*, Cathepsins B, H, L and cysteine protease

inhibitors in malignant prostate cell lines, primary cultured prostatic cells and prostatic tissue. *Eur J Cancer* 1999, 35, 138-144.

27. Adams, D., Larman, B., and Oxburgh, L., Developmental expression of mouse follistatin-like 1 (Fstl1): Dynamic regulation during organogenesis of the kidney and lung. *Gene. Expr Patterns 2007,* 7, 491-500.

28. Joesting, M. S., Perrin, S., Elenbaas, B., Fawell, S. E.*, et al.*, Identification of SFRP1 as a candidate mediator of stromal-to-epithelial signaling in prostate cancer. *Cancer Res* 2005, 65, 10423-10430.

29. Cunha, G. R., Cooke, P. S. and Kurita, T., Role of stromal-epithelial interactions in hormonal responses. *Arch Histol Cytol* 2004, 67, 417-434.

30. Sainaghi, P. P., Castello, L., Bergamasco, L., Galletti, M.*, et al.*, proliferation in prostate carcinoma cell lines expressing the Axl receptor. *J Cell Physiol* 2005, 204, 36-44.

31. Gorringe, K. L., Chin, S. F., Pharoah, P., Staines, J. M.*, et al.*, Evidence that both genetic instability and selection contribute to the accumulation of chromosome alterations in cancer. *Carcinogenesis* 2005, 26, 923-930.

32. Leung, K. C., Brce, J., Doyle, N., Lee, H. J.*, et al.*, Regulation of growth hormone signaling by selective estrogen receptor modulators occurs through suppression of protein tyrosine phosphatases. *Endocrinology* 2007, 148, 2417-2423.

33. Olsen, J. V., Blagoev, B., Gnad, F., Macek, B.*, et al.*, Global, in vivo, and site-specific phosphorylation dynamics in signaling networks. *Cell* 2006, 127, 635-648.

34. Wesley, U. V., McGroarty, M. and Homoyouni, A., Dipeptidyl peptidase inhibits malignant

phenotype of prostate cancer cells by blocking basic fibroblast growth factor signaling pathway. *Cancer Res* 2005, 65, 1325-1334.

35. McNeil, H. P., Simpson, R. J., Chesterman, C. N. and Krilis, S. A., Anti-phospholipid antibodies are directed against a complex antigen that includes a lipid-binding inhibitor of coagulation: beta 2-glycoprotein I (apolipoprotein H). *Proc Natl Acad Sci U S A* 1990, 87, 4120-4124.

36. Kang, H. R., Yim, E. Y., Oh, S. Y., Chang, Y. S., *et al.*, Normal C1 inhibitor mRNA expression level in type I hereditary angioedema patients: newly found C1 inhibitor gene mutations. *Allergy* 2006, 61, 260-264.

37. Trojan, L., Schaaf, A., Steidler, A., Haak, M., *et al.*, Identification of metastasis-associated genes in prostate cancer by genetic profiling of human prostate cancer cell lines. *Anticancer Res* 2005, 25, 183-191.

38. Chung, L. W., Huang, W. C., Sung, S. Y., Wu, D., *et al.*, Stromal-epithelial interaction in prostate cancer progression. *Clin Genitourin Cancer* 2006, 5, 162-170.

ACKNOWLEDGMENT

This work was supported in full by Department of Army grant W81XWH-06-1-0108; and in part by National Institutes of Health grants: CA101052, DK63630; UW NIEHS sponsored Center for

Ecogenetics and Environmental Health (NIEHS P30ES07033); and NW RCE for Biodefense and Emerging Infectious Diseases (1U54 AI57141).

FIGURE LEGENDS

Figure 1. Expression of stromal cell-specific marker by RT-PCR. Cultured prostate and bladder stromal cells were harvested and analyzed by RT-PCR for expression of αSMA – positive and EGP - negative. EGP expression was detected in LNCaP cells

Figure 2. Secreted proteomes of cultured prostate and bladder stromal cells. The Venn diagram shows the overlap of the two proteomes, PS = prostate, BL = bladder. Altogether, 118 proteins were identified.

Figure 3. Gene Ontology subcellular categorization of the proteomes. Most of the identified secreted proteins, 76%, were categorized as extracellular or membrane-associated. NA = not available.

Figure 4. Gene Ontology molecular function distribution. Overall, a similar pattern is seen for the two proteomes but with protein binding and hydrolase activity being more prominent in the prostate group.

Figure 5. Gene Ontology biological process distribution. Prominent among the processes identified are physiology and communication.

Figure 6. Western blot analysis of identified differentially expressed proteins. Protein preparations of cultured cells were resolved by gel electrophoresis and probed by antibodies to gene products listed on the right. CD90 served as the control for sample loading. The expression level as indicated by the band intensity correlated well with the PICA quantification method.

Figure 7. ECM-receptor interaction pathway. Six secreted proteins involved in the pathway were identified by in this study. Pathway was analyzed and visualized by Teranode. Protein expression is indicated by the relative expression level of the prostate over bladder. LAMC1 and FN1, with increased expression in prostate stromal cells, are colored green; LAMB1 and LAMA4, with similar levels of expression in the two cell types, are colored yellowish green and yellow; THBS1 and ARGN, with no expression ratio since they were found only in the prostate, are colored turquoise.

TABLES

Table 1. Differentially expressed secreted proteins identified from the cultured prostate and bladder stromal cells. The protein IPI ID, ProteinProphet probability (PB) score, annotation, expression ratio, false discovery rate, peptide sequence (with putative glycosylation site underlined), GO biological process, GO molecular process, and GO cellular compartment information are tabulated. The list is sorted by FDR.

Protein	PB	Annotation	PS/BL	FDR	Peptides	Biological process	Molecular function	Cellular location
IPI00012887	1	Cathepsin L	4.9	0.001	YSVANDTGFVDIPKQEK YSVANDTGFVDIPK	physiological process	hydrolase activity	extracellular
IPI00029723	1	Follistatin-related protein 1	11.0	0.001	GS**NYS**EILDK FVEQ**NET**AI**NIT**TYPDQENNK VEQ**NET**AI**NIT**TYPDQENNK	NA	carbohydrate binding	extracellular
IPI00297160	1	Hypothetical protein DKFZp451K1918	3.7	0.001	AF**NST**LPTMAQMEK	cell adhesion	carbohydrate binding	membrane associated
IPI00029131	1	Neuroendocrine convertase 2	3.0	0.001	RGDLNI**NMT**SPMGTK RNPEAGVATTDLYG**NCT**LR NPEAGVATTDLYG**NCT**LR	physiological process	hydrolase activity	extracellular
IPI00015657	1	Pregnancy-specific beta-1-glycoprotein 5	9.9	0.001	ILILPSVTR**NET**GPYECEIR	physiological process	NA	extracellular
IPI00298281	1	Laminin gamma-1 chain	7.6	0.001	LLN**NLT**SIK V**NNT**LSSQISR TA**NDT**STEAYNLLLR	positive regulation of biological process	extracellular matrix structural	membrane associated
IPI00020986	1	Lumican	2.6	0.001	LHINHN**NLT**ESVGPLPK KLHINHN**NLT**ESVGPLPK	physiological process	protein binding	extracellular
IPI00339227	1	Splice Isoform 7 Of Fibronectin	7.5	0.002	DQCIVDDITYNV**NDT**FHK WTPL**NSS**TIIGYR HEEGHML**NCT**CFGQGR	cell adhesion	protein binding	extracellular
IPI00296992	0.99	AXL receptor tyrosine kinase, isoform 1	7.0	0.002	EESPFVGNPG**NIT**GAR	physiological process	transferase activity	membrane associated
IPI00298362	1	Tumor necrosis factor receptor superfamily member 11B	3.9	0.002	HIGHA**NLT**FEQLR KHT**NCS**VFGLLLTQK CPDGFFS**NET**SSK	development	protein binding	extracellular
IPI00014572	1	SPARC	7.2	0.002	VCSND**NKT**FDSSCHFFATK	development	ion binding	extracellular
IPI00009198	1	Tissue factor pathway inhibitor 2	4.0	0.002	DEGLCSA**NVT**R YFF**NLS**SMTCEK	coagulation	extracellular matrix structural	extracellular
IPI00328113	1	Fibrillin 1	7.4	0.002	TAIFAF**NIS**HVSNK	development	ion binding	extracellular
IPI00018305	0.99	Insulin-like growth factor binding protein 3	10.9	0.003	GLCV**NAS**AVSR	positive regulation of biological process	enzyme	extracellular
IPI00419941	1	PTK7 protein tyrosine kinase 7, isoform a	1.7	0.003	MHIFQ**NGS**LVIHDVAPEDSGR	physiological process	transferase activity	membrane associated
IPI00021081	1	Splice Isoform 1 Of Follistatin	2.2	0.003	SDEPVCASD**NAT**YASECAMK	development	protein binding	extracellular
IPI00032292	1	Metalloproteinase inhibitor 1	2.1	0.004	SH**NRS**EEFLIAGK FVGTPEV**NQT**TLYQR AKFVGTPEV**NQT**TLYQR VGTPEV**NQT**TLYQR	positive regulation of biological process	enzyme	extracellular
IPI00298828	1	Beta-2-glycoprotein I	0.2	0.006	VYKPSAG**NNS**LYR LG**NWS**AMPSCK	cell communication	carbohydrate binding	extracellular
IPI00291866	1	Plasma protease C1 inhibitor	0.5	0.006	DTFV**NAS**R NP**NAT**SSSSQDPESLQDR	physiological process	enzyme	extracellular
IPI00470937	1	Protein tyrosine phosphatase, receptor type, K	3.8	0.006	GPLANPIW**NVT**GFTGR	physiological process	hydrolase activity	membrane associated
IPI00289819	1	Cation-independent mannose-6-phosphate receptor	1.5	0.008	DAGVGFPEYQEED**NST**YNFR	physiological process	transferase activity	membrane associated
IPI00022810	1	Dipeptidyl-peptidase I	63.9	0.008	DV**NCS**VMGPQEK VTTYC**NET**MTGWVHDVLGR	cell communication	hydrolase activity	intracellular
IPI00169285	1	Hypothetical protein LOC196463	0.1	0.021	HPDAVAWA**NLT**NAIR	NA	NA	NA
IPI00023673	1	Galectin-3 binding protein	3.5	0.024	YKGL**NLT**EDTYKPR DAGVVCT**NET**R AAIPSALDT**NSS**K ALGFE**NAT**QALGR	cell communication	signal transducer	extracellular
IPI00218019	0.99	Splice Isoform 1 Of Basigin	1.8	0.038	ILLTCSL**NDS**ATEVTGHR	cell communication	carbohydrate binding	membrane associated
IPI00016112	1	Melanoma associated gene	11.7	0.045	QGEHLS**NST**SAFSTR ILCDNAD**NIT**R LSTTECVDAGGESHA**NNT**K	cell communication	protein binding	NA

IPI00339223	1	Splice Isoform 3 Of Fibronectin	6.4	0.046	HEEGHML**NCT**CFGQGR RHEEGHML**NCT**CFGQGR DQCIVDDITYNV**NDT**FHK	cell adhesion	protein binding	extracellular
IPI00028931	0.98	Desmoglein 2	0.7	0.050	I**NAT**DADEPNTLNSK	cell adhesion	ion binding	membrane associated
IPI00003813	1	Nectin-like protein 2	0.7	0.050	VSLT**NVS**ISDEGR FQLL**NFS**SSELK DTAVEGEEIEV**NCT**AMASK	cell adhesion	protein binding	membrane associated

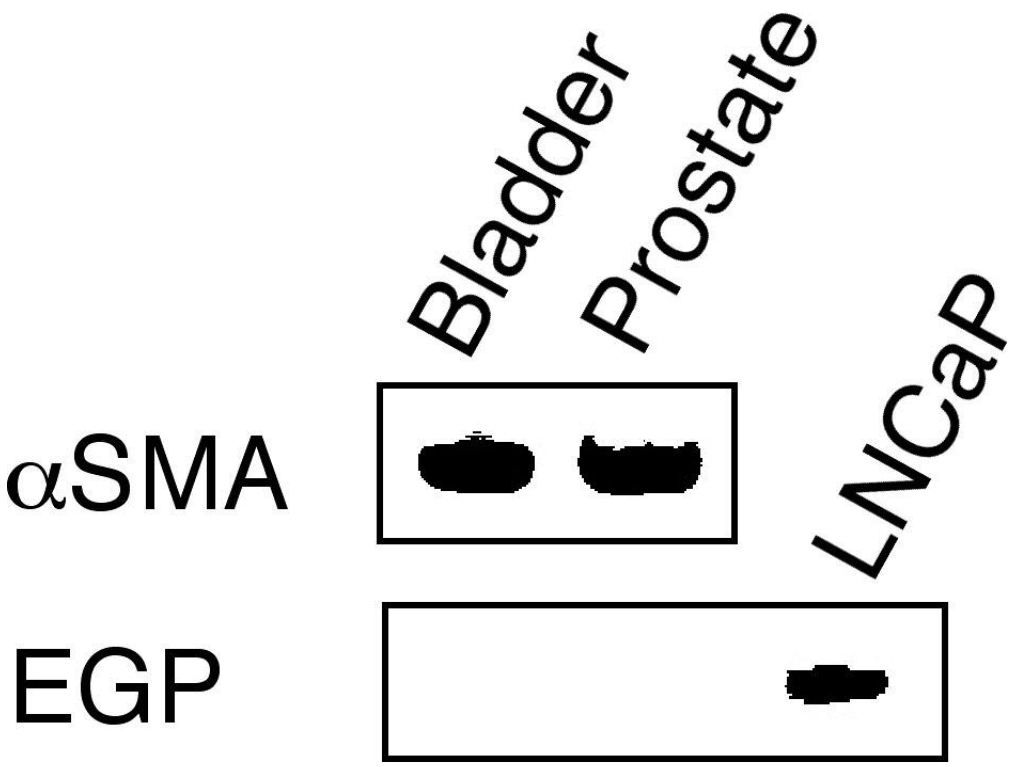
Table 2. Pathway distribution of identified secreted proteins. KEGG pathway ID number, pathway name, number of identified proteins in the pathway, and gene ID are listed in columns 1 to 4 respectively.

KEGG_ID	Pathway (*Homo sapiens*)	Protein number	GENE ID
KEGG_hsa04512	ECM-receptor interaction	6	LAMA4, LAMB1, LAMC1, FN1, THBS1, AGRN
KEGG_hsa04610	Complement and coagulation cascades	6	A2M, PLAU, SERPINC1, MASP2, CD59, SERPING1
KEGG_hsa04510	Focal adhesion	6	LAMA4, LAMB1, LAMC2, FN1, THBS1, HGF
KEGG_hsa04514	Cell adhesion molecules (CAMs)	5	ALCAM, PVR, NEO1, CNTN1, NRCAM
KEGG_hsa04360	Axon guidance	4	EFNA1, EFNA5, SEMA7A, PLXNB2
KEGG_hsa04810	Regulation of actin cytoskeleton	2	CD14, FN1
KEGG_hsa05060	Prion disease	2	LAMB1, LAMC1
KEGG_hsa05010	Alzheimer's disease	2	LRP1, A2M
KEGG_hsa04060	Cytokine-cytokine receptor interaction	2	TNFRSF11B, HGF

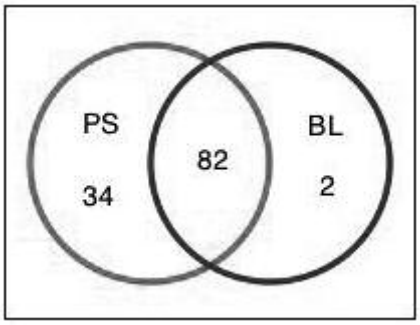
SUPPORTING INFORMATION

Supplemental_table1.pdf :

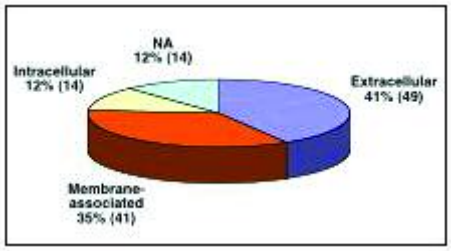
Secreted proteins identified from cultured prostate and bladder stromal cells. The protein IPI ID, ProteinProphet probability, annotation, expression type, expression ratio (prostate/bladder), FDR, peptide sequence (with glycosylation site motif underlined), number of unique peptide identified, GO biological process, GO molecular process, and GO cellular compartment are listed from columns 1 to 10 respectively. The list is sorted by FDR.



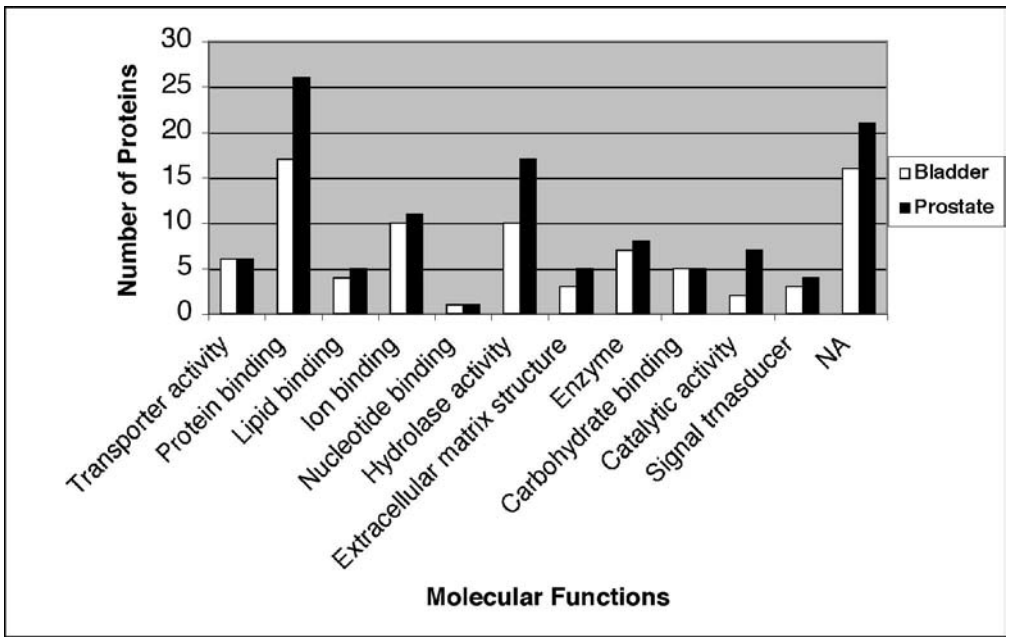
41x31mm (576 x 576 DPI)



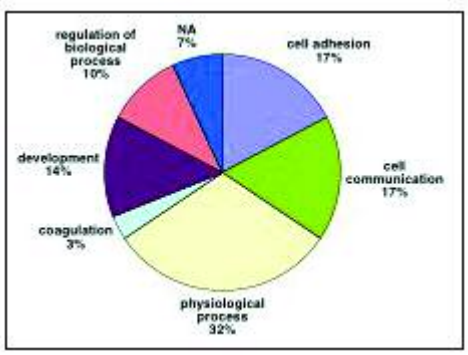
75x58mm (72 x 72 DPI)



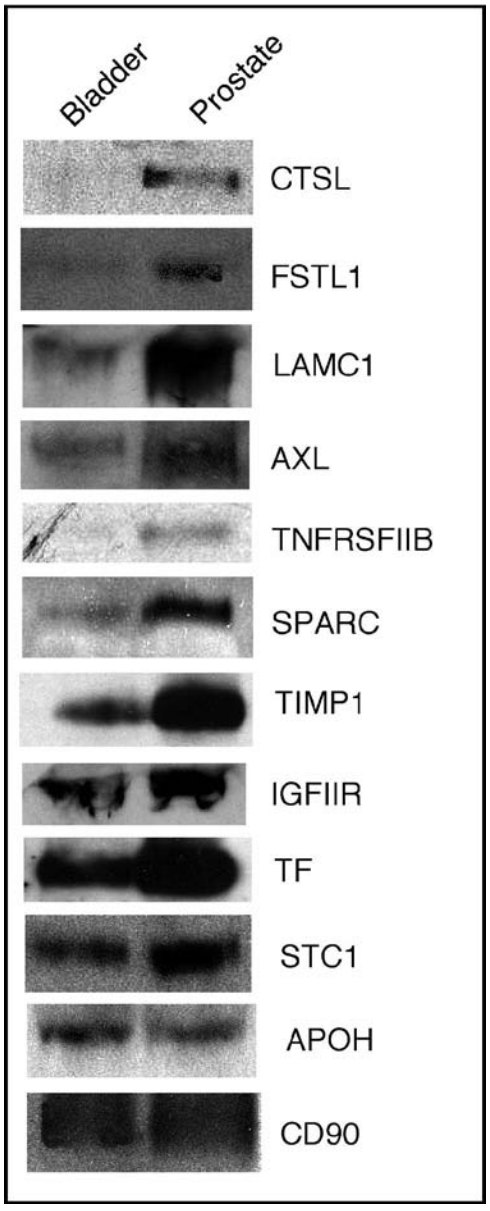
81x45mm (72 x 72 DPI)



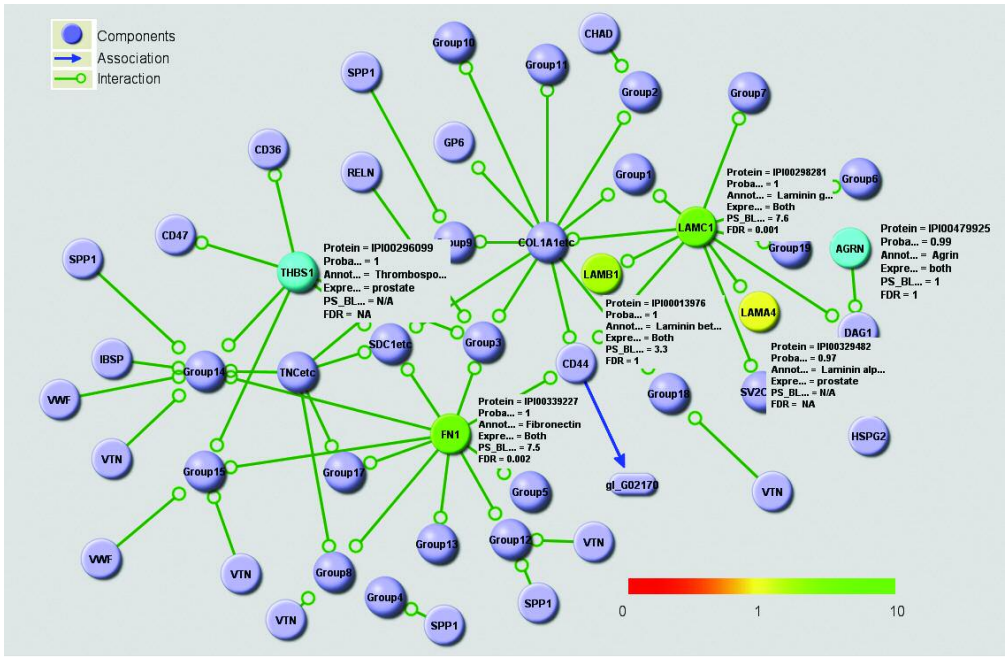
84x53mm (576 x 576 DPI)



83x63mm (72 x 72 DPI)



65x163mm (300 x 300 DPI)



95x62mm (300 x 300 DPI)

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel on page 1 of the Detailed Cost Estimate form for the initial budget period.

NAME	POSITION TITLE
Young Ah Goo	Postdoctoral Fellow

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
ChonNam National University	B.Sc.	1990	Biology
University of Washington	M.Sc.	1994	Pathobiology
University of Washington	Ph.D.	2002	Pathobiology/Genome Sciences
Institute for Systems Biology	Post-doctorate	2002-2004	Prostate research/Proteomics
University of Washington	Post-doctorate	2004 -	Prostate research/Proteomics

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past 3 years and representative earlier publications pertinent to this application. PAGE LIMITATIONS APPLY. DO NOT EXCEED FOUR PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INVESTIGATOR.

A. Positions and Honors

Positions and Employment

1991-1992 Research Technologist, ChonNam National University, South Korea
1992-1994 Graduate Research Assistant, Pathobiology, University of Washington (UW), Seattle, WA
1995-1996 Graduate Research Fellow, Pathobiology, UW, Seattle, WA
1997 President, Korean Biologist meeting in Seattle (KBMS), Seattle, WA
1997-2002 Graduate Research Assistant, Genome Science, UW, Seattle, WA
2002-2004 Post-Doctoral Fellow, Institute for Systems Biology, Seattle, WA
2003- Invited journal reviewer
- Proteomics
- Cancer Informatics
- Molecular Cellular Proteomics
- Rapid Communications in Mass Spectrometry

2004- Post-Doctoral Fellow, Medicinal Chemistry, UW, Seattle, WA

Awards and Fellowships

1987-1990 Honor Student Scholarship. ChonNam National University, S. Korea
1990 B.Sc. with summa cum laude. ChonNam National University, S. Korea
1995 Traveling Funds Award for National ASM Conference. American Society for Microbiology (ASM)
2000 Traveling Funds Award for International ASM Conference.
1997-2002 NSF Research Assistant Fellowship for Ph.D. Study. Genome Science, UW
2004 Young Scientist Award. The Human Proteome Organization (HUPO). Beijing, China
2005-2007 Department of Defense Prostate cancer research fellowship. Department of Medicinal Chemistry, University of Washington, Seattle, WA

B. Publications (past 3 years, chronological order)

Goo, Y. A.; Goodlett, D. R.; Pascal, L. E.; Worthington, K. D.; Vessella, R. L.; True, L. D.; Liu, A. Y., Stromal mesenchyme cell genes of the human prostate and bladder. *BMC Urol* **2005,** 5, 17.

Deutsch, E. W.; Ball, C. A.; Bova, G. S.; Brazma, A.; Bumgarner, R. E.; Campbell, D.; Causton, H. C.; Christiansen, J.; Davidson, D.; Eichner, L. J.; **Goo, Y. A**. *et al.,* Development of the Minimum Information Specification for In Situ Hybridization and Immunohistochemistry Experiments (MISFISHIE). *Omics* **2006,** 10, (2), 205-8.

Goo, Y. A.; Pajkovic, N.; Shaffer, S.; Taylor, G.; Chen, J.; Campbell, D.; Arnstein, L.; Goodlett, D. R.; van Breemen, R., Systematic investigation of lycopene effects in LNCaP cells by use of novel large-scale proteomic analysis software. *Proteomics-Clin Appli* **2007**, 1, (50), 513-23.

Pascal, L. E.; Oudes, A. J.; Petersen, T. W.; **Goo, Y. A**.; Walashek, L. S.; True, L. D.; Liu, A. Y., Molecular and cellular characterization of ABCG2 in the prostate. *BMC Urol* **2007,** 7, 6.

Ryu, S.; Gallis, B.; **Goo, Y. A**.; Shaffer, S. A.; Radulovic, D.; Goodlett, D. R., Comparison of a Label- free quantitative proteomic method based on peptide ion current area to the isotope coded affinity tag method. *In press*

Goo, Y. A.; Liu, A. Y.; Ruy, S.; Shaffer, S.; Malmstroem, L.; Page, L.; Nguyen, L. T.; Doneanu, C. E.; Goodlett, D. R., Identification of secreted glycoproteins of human prostate and bladder stromal cells by comparative quantitative proteomics. *Submitted*

C. Poster Presentations (past 3 years, chronological order)

Goo, YA., Pajkovic, N., Ryu, S., Chen, J., Shaffer, S., Radulovic, D., van Breemen, RB., and Goodlett, DR. Effects of lycopene treatment on the sub-cellular proteomes of human LNCaP cells. European Symposium of The Protein Society. April 2005. Barcelona, Spain

Goo, YA., Goodlett, DR., Pascal, LE., Worthington, KD., Vessella, RL., True, LE., and Liu, AY. Proenkephalin (PENK): a candidate stromal mesenchyme cell gene associated with the prostate-specific differentiation and cancer development. American Urologic Association Annual Meeting. May 2005. San Antonio, TX

Goo, YA., Zheng, L., Pajkovic, N., Taylor, G., Arnstein, L., Shaffer, S., van Breemen, RB., and Goodlett, DR. Systematic investigation of lycopene mechanism in prostate cancer using novel large-scale proteomic analysis software. 54th ASMS (American Society for Mass Spectrometry) Conference. May 2006. Seattle, WA

Goo, YA., Ryu, S., Walashek, L., Shaffer, S., Liu, AY., Goodlett, DR. Comparative analysis of secreted proteins of human prostate and bladder stromal mesenchyme cells. 7th Siena Meeting: From Genome to Proteome. Siena, Italy Oct 2006, Poster presentation

Goo, YA., Ryu, S., Shaffer, S., Walashek, L., Liu, AY., Goodlett, DR. Secreted glycoproteins of human prostate and bladder stromal mesenchyme cells. USHUPO 3rd Annual Conference. Seattle, USA Mar 2007, Poster presentation

Goo, YA., Liu, AY., Goodlett, DR. Identification of human prostate and bladder stromal factors by quantitative transcriptome and proteomics analysis. CDMRP PCRP 2007 IMPaCT Meeting. Atlanta, USA Sep 2007, Poster presentation

Goo, YA., Liu, AY., Goodlett, DR. Identification of human prostate and bladder stromal cell signaling factors by quantitative proteomic and transcriptome analysis. HUPO 6th Annul World Congress. Seoul, Korea Oct 2007, Poster presentation

D. Oral Presentations

Goo, YA., Stromal mesenchyme cell genes associated with the organ-specific differentiation of epithelial cells and cancer development. Dept. of Urology University of Washington. June 2004

Goo, YA., Proenkephalin (PENK): a candidate stromal mesenchyme cell gene associated with the prostate-specific differntiation and cancer. Dept. of Urology, University of Washington. June 2005

Goo, YA., Identification of candidate stromal mesenchyme cell factors associated with prostate- and bladder-specific epithelial differentiation. Dept. of Urology, University of Washington. Mar. 2007

Goo, YA., Identification of secreted glycoproteins of human prostate and bladder stromal cells by comparative quantitative proteomics. Dept. of Urology, University of Washington. June 2007